Water, Microorganisms and Volatile Bases in Dehydrated Fish

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ABSTRACT

The relations of relative humidity (R.H.) to water content in precooked, air-dehydrated fish were determined with reference to the mould, bacterial and volatile-base content of stored samples. In general it was found that visible mould developed only in samples stored at 80% or higher R.H., while bacteria increased only at 85% or higher R.H. Viable bacteria and moulds decreased slowly in samples stored at R.H. too low to support their active multiplication. Total volatile bases and trimethylamine increased in samples stored at R. H. too low for growth of either bacteria or moulds.

Artificial dehydration of fish is by no means a recent innovation, for as long ago as 1877 and 1880 patents were issued to Alden for methods of preparing precooked dehydrated fish (cited by Stevenson 1899). The possibility of utilizing dehydrated fish as a convenient economical source of animal protein in human nutrition was recognized during the war of 1914 to 1918, for about that time methods of preparing both raw and precooked dehydrated fish were described (Duge 1913, cited by Tressler 1923; Falk, Frankel and McKee 1919). It is not clear whether any of the processes described were applied commercially. During the present emergency renewed interest has been taken in this type of product. Cutting and Reay (1944), using equipment previously described by Cutting (1942), showed that precooked dehydrated fish was easier to produce than raw dehydrated fish, though Kidd (British Patent 539,477 in 1938) had previously produced an apparently satisfactory raw dehydrated fish by drying the minced frozen flesh under fairly high vacuum ("Vac-ice" process). In general, most workers appear to agree that the best practical results are obtained by precooking whole gutted fish in steam, flaking the flesh from the bones (the cooked flesh may be pressed to remove some of the fat and excess watery liquid), mincing, and drying in a stream of warm air (Young and Sidaway 1943; Young and Lee 1943; Martinek and Jacobs 1943; Cutting and Reay 1944). The equipment and individual methods used by different investigators have varied, but it appears to be generally agreed that dehydration of the minced cooked flesh should be completed in a few hours and that the temperature of the product should not rise above 60 or 70°C. during processing. The present investigation was undertaken with a view to determining some of the conditions necessary for satisfactory storage of precooked dehydrated fish; a preliminary report has already been published (Tarr 1943).

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EXPERIMENTAL

Only fresh fish were used. These were precooked and, with one exception, dehydrated by the process developed at this Station (Young and Sidaway 1943). In general a temperature range of 50 to 60°C. was employed in the dehydration, care being taken not to overheat the fish. It was assumed that minor variations in temperature and time employed in dehydration did not affect the results of the experiments to be described. Viable bacteria and mould and yeast counts were made by the roll tube method (Tarr and Bailey 1939), the dehydrated fish samples being ground mechanically with sand for 5 minutes with fourteen times their weight of sterile water prior to analysis. Double strength Bacto nutrient agar containing 0.02 M phosphate buffer (pH 7) was employed for the bacterial counts. The same medium containing added glucose and tartaric acid in the proportions described by Henrici (1930) was usually employed for mould and yeast counts. In general, the ordinary medium used favoured growth of bacteria, while the glucose-tartaric acid medium favoured growth of moulds and yeasts. However, both yeasts and moulds occasionally developed on the former, and yeasts, and possibly certain bacteria, on the latter, so that the differentiation was not very sharp. In the experiments to be described all colonies, except obvious moulds, which developed on the ordinary medium were recorded as "viable bacteria," while all those which developed on glucose-tartaric acid medium were recorded as "moulds," though with certain samples numerous yeasts developed on this medium. In some instances where large numbers of mould colonies grew on the ordinary medium they were counted and the results recorded separately. Total volatile base (probably largely ammonia plus trimethylamine) and trimethylamine determinations were made on aqueous extracts, previously prepared for determining the number of microörganisms, using the Conway dish technique (Tarr and Bailey 1939). This method, which employs formaldehyde to bind ammonia and monomethylamine, determines trimethylamine and dimethylamine, so that the figures given for trimethylamine in this paper include any dimethylamine which may have been present in the samples. Constant humidity experiments were carried out in small glass desiccators, or similar vessels, containing 200 ml. of a solution of either calcium chloride (Macara 1943) or sulphuric acid of a concentration calculated to maintain the desired relative humidity (R.H.) at 25°C. Samples of dehydrated fish weighing about 10 g. were placed in 9-cm. sterilized open petri dishes on glass tripods over these solutions, the lids of the containers being sealed with a heavy grease made of vaseline and paraffin wax prior to storing them.

Water content was determined on approximately 2-g. samples of dehydrated fish by exposing them to a temperature of 100 to 101°C. for 24 hours in an air oven, cooling in the presence of phosphorus pentoxide, and weighing. In this connection it is of interest to note that moisture has been determined in dehydrated meat with apparently satisfactory results by distillation with toluene (Bate-Smith, Lea and Sharp 1943), and by drying to constant weight over phosphorus pentoxide at 30°C. or by drying for 6 hours at 100°C. in an air oven (Gane 1943). Drying in partial vacuum at 100°C. to constant weight has also been recommended (A.O.A.C. Official Methods of Analysis 1925). In the writer's experience samples of dehydrated fish do not attain absolutely constant

weight after 24 hr. at 100°C. in an air oven, but continue to lose a small amount of weight very slowly for many days when kept at 100°C. They simultaneously become very dark in colour.

Alterations in Microbiological Content

Determinations of the numbers of bacteria and of yeasts and moulds per gram were made on samples of herring (*Clupea pallasii*), lingcod (*Ophiodon elongatus*), coho salmon (*Oncorhynchus kisutch*) and chum salmon (*Oncorhynchus keta*) at different stages in the dehydration process, namely (*a*) whole fish after precooking, (*b*) minced fish, and (*c*) dehydrated fish. The bacteria were as follows: herring,—(*a*) 14, (*b*) 720, (*c*) 17,000; lingcod,—(*b*) 96, (*c*) 930; coho salmon,—(*b*) 820, (*c*) 4,700; chum salmon,—(*a*) 0, (*b*) 79 and 390, (*c*) 23,000 and 1,800. The moulds were: lingcod,—(*b*) 8, (*c*) 22; coho salmon,—(*b*) 8, (*c*) 60; chum salmon,—(*a*) 0, (*b*) 3 and 8, (*c*) 380 and 45. There was thus a progressive increase in bacteria and moulds during mincing and dehydration of the cooked flesh. Factors such as contamination during mincing, concentration of organisms due to shrinkage of the material during dehydration, aerial contamination and, possibly, growth of organisms during the early stages of dehydration, were probably responsible for this.

Nineteen colonies which developed on ordinary medium in roll tubes from seven different samples of dehydrated fish, some freshly prepared and some after storage for three months, were examined microscopically. Of these, sixteen were cocci or very short fat rods almost coccoid in shape, two were yeasts and one was a spore-forming rod. Small gram-negative rods of the *Achromobacter* or *Pseudomonas* type were not found.

Lingcod and Coho Salmon Stored Over $CaCl_2$

The sample of dehydrated lingcod used differed from the other samples of dehydrated fish employed in this work in that the minced cooked flesh was dried in a rather thick layer in a very slow stream of air for about a day at 45 to 50°C. These conditions favoured bacterial growth as is shown by the high initial bacterial content (table I), while the dried product was hard and did not possess the crispness of samples prepared in the usual manner. The dehydrated lingcod contained 77%, and the coho salmon 83% protein. Samples were stored at 25°C. over calcium chloride solutions at varied R.H.; the development of visible mould was recorded and viable bacterial counts and water determinations were made at stated intervals. It will be seen from table II that visible mould developed in samples stored only at 80% or higher R.H., corresponding to a water content of 17.2% and 17.9% of coho and lingcod respectively. Bacteria increased very rapidly at 90 and 100% R.H. At 85% R.H., af er 18 days there was an apparent increase beyond the initial bacterial content of the coho salmon, whereas after the same period there was a marked decrease from the initial high bacterial content of the lingcod. Viable bacteria decreased rapidly at first (as between initial values and those after 15 days' storage) and then more slowly (as between 45 and 112 days) in samples stored at from 65 to 80% R.H. In the sample of coho salmon stored for 112 days at 75% R.H. a mould count much higher than that found in certain samples of freshly prepared dehydrated

TABLE I. Effect of storing over calcium chloride solutions at different relative humidities on mould development, water and bacterial content of dehydrated lingcod and coho salmon.

	Species of fish	Initial values	Relative humidity (%)						
			65	70	75	80	85	90	100
Time before appearance of mould (days).	Coho salmon		*	*	*	34**	18	9	7
	Lingcod		*	*	*	52**	18	9	7
Water content at time of first appearance of mould (%). In samples in which visible mould did not develop, the water content was determined after 45 days' storage.	Coho salmon	4.8	11.8	12.8	13.5	17.2	19.2	27.6	29.4 (37.5)†
	Lingcod	2.6	12.2	13.1	14.1	17.9	19.7	30	28.3 (37.3)†
Water content after 112 days' storage (%).	Coho salmon	4.8	11.5	12.4	13.5	16.6			
	Lingcod	2.6	12.4	13.0	14.3	18.3			
Viable bacteria at first appearance of mould (colonies per g.). In samples in which visible mould did not develop, this determination was made after 45 days' storage.	Coho salmon	13,000	53	140	410	120	86,000	180×10^6	440×10^6
	Lingcod	16×10^6	110,000	190,000	37,000	240,000	1.5×10^6	250×10^6	370×10^6
Viable bacteria after 112 days' storage (colonies per g.)	Coho salmon	13,000	38	15	0	0			
	Lingcod	16×10^6	25,000	41,000	19,000	0			
Viable moulds after 112 days' storage (colonies per g.). These developed and were counted on the medium used for determining numbers of viable bacteria.	Coho salmon		300	75	1,500	4,700			
	Lingcod		0	0	0	2,600			

*No visible mould developed after 112 days' storage.

**Only one small patch of mould developed.

†The values given in brackets were obtained after 12 days' storage when the samples were extremely mouldy.

fish was found. Unfortunately initial mould counts were not made on the freshly dehydrated fish so that there was no definite means of knowing whether the observed rather high count was due to actual growth of mould, though the results of the next experiment indicated that some mould growth occurs at this humidity.

CHUM SALMON STORED OVER $CaCl_2$

This experiment was carried out under conditions similar to those described in the foregoing one with the exception that total volatile base and trimethylamine determinations were made. The results are given in table II. As far as the relation between R.H., water, bacterial content and visible growth of mould were concerned the results were, in general, closely similar to those obtained in

TABLE II. Effect of storing over calcium chloride solutions at different relative humidities on mould development and water, bacterial and volatile base content of dehydrated chum salmon. A is value obtained either at first appearance of mould or, in those samples in which visible mould did not develop, after 35 days' storage; B is value obtained after 102 days' storage.

		Initial values	Relative humidity						
			65	70	75	80	85	90	100
Time before first appearance of mould (days)			*	*	*	35 †	17	8	6
Water content (%)	A	4.1	11.0	12.1	13.6	16.8	19.0	23.2	33.1
	B	4.1	10.8	11.9	13.2	16.7			
Viable bacteria (colonies per g.)	A	23,000	5,300	12,000	15,000	61,000	1,100	7.6×10^6	67×10^6
	B	23,000	225	500,000	150	570			
Viable moulds developing on the medium used for bacterial counts (colonies per g.)	A	380	0	0	0	120,000	51,000	2.6×10^6	140×10^6
	B	380	165	0	64,000	>100,000			
Total volatile base (mg. N per 100 g.)	A	38	66	77	53	46	22	9.1	37
	B	38	82	81	48	26			
Trimethylamine (mg. N per 100 g.)	A	0	12	12	8.5	8.9	4.2	1.9	5.3
	B	0	27	33	17	22			

*No visible mould developed in 102 days.
†Only a moderate growth of mould occurred and this sample was stored for 102 days.

the foregoing experiment. The rather low bacterial count obtained in the sample stored for 17 days at 85% R.H., and the exceptionally high bacterial count obtained in the sample stored for 102 days at 70% R.H. are difficult to explain, though it is not unlikely that sampling errors were responsible. Similar irregularities in bacterial content were observed in the samples studied in the experiment next to be described. The increase in the mould count obtained in the sample stored at 75% R.H. showed that mould may grow on dehydrated fish and yet not to a sufficient extent to be visible to the naked eye. Except in the case of the sample stored at 70% R.H. there was a marked decrease in the viable bacteria in all cases after 102 days' storage. There were marked changes in the volatile base content of the stored samples. At humidities at which growth of bacteria and moulds occurred these changes could be due to formation of volatile bases (especially by bacteria), or to their utilization by the microörganisms. Volatilization of these basic compounds might also account for certain of the observed losses. There was a pronounced increase in the total volatile base content of the sample stored at 65% R.H., at which value the numbers of viable organisms had decreased. Trimethylamine developed in all samples, but most markedly in those stored at the lower humidities corresponding to moisture contents of about 11% and 12%.

Dehydrated lingcod was stored over sulphuric acid at between 10 and 80% R.H., analyses being made at given intervals. The results are recorded in table

TABLE III. Effect of storing over sulphuric acid solutions at different relative humidities on mould development and moisture, bacterial and volatile base content of dehydrated lingcod.

	Time of storage (days)	Initial values	Relative humidity (%)							
			10	20	30	40	50	60	70	80
Water content (%)	30	1.7	3.0	4.5	5.7	6.8	8.3	9.3	13.6	17.9
	97	1.7	3.2	4.3	5.5	7.2	8.5	9.2	13.4	16.8
Viable bacteria (colonies per g.)	30	4,700	7,400	4,900	6,300	9,800	66,000	4,100	4,600	900
	97	4,700	12,000	2,700	2,600	3,200	2,600	1,100	320	*
Total volatile base (mg. N per 100 g.)	30	27	24	24	29	28	28	28	12	11
	97	27	22	23	30	33	34	34	21	20
Trimethylamine (mg. N per 100 g.)	30	4.2	2.2	4.2	8.4	8.4	12.6	4.8	4.8	3.1
	97	4.2	8.9	8.9	13	25	24	22	16	14

*Visible mould appeared after 76 days' storage. No bacterial colonies developed in the roll tubes, probably because their development was suppressed by the moulds which were very numerous.

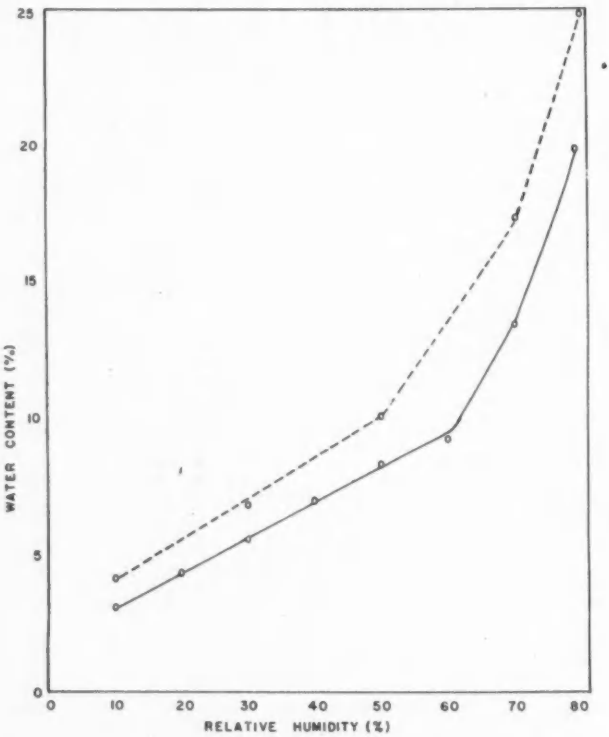

FIGURE 1. Relation between relative humidity and water content of precooked, air-dehydrated lingcod stored at 25°C. (continuous line) and of precooked, air-dehydrated beef stored at 37°C. (broken line; data taken from paper by Gane, 1943; water content calculated on fat-free basis).

III, the relation between R.H. and water content being shown in figure 1. It was found that the water content increased linearly with increasing humidity at 10 to 60% R.H., but that at higher humidities this relation was no longer maintained, the water content increasing sharply. In this respect the results parallel closely those obtained by Gane (1943) for pre-cooked, air-dehydrated beef (figure 1). There was slow development of visible mould at 80% R.H. (average moisture content 17.4%), and in this respect the results were identical with those obtained in the two previous experiments. If it is assumed that the high bacterial count obtained in the sample stored for 30 days at 50% R.H. was due to sampling error, it would appear that bacteria in dehydrated fish stored at 10 to 50% R.H. decrease only slightly, but that at higher humidities the decrease is more marked. The total volatile base had not increased in samples held for 30 days at 10 to 60% R.H.; there was, however, a definite decrease at 70 and 80% R.H. After 97 days only a slight increase in total volatile base was found at 30 to 60% R.H., while there was a slight decrease at 10, 20, 70 and 80% R.H. In this respect the results differed from those obtained in the foregoing experiment, and it is suggested that volatilization of ammonia and its neutralization by the sulphuric acid, over which the samples were stored, may have accounted for the failure of total volatile base to accumulate. The trimethylamine content of most samples increased after storage for 30 days, while after 97 days there was a marked increase in all cases, the accumulation being greatest at 40 and 50% R.H. Apparently under the conditions of storage trimethylamine did not volatilize as readily as ammonia.

Lingcod, Chum Salmon and Coho Salmon Stored in Sealed Cans

The water, volatile base and bacterial content of several samples of dehydrated fish were determined. Portions were then filled into ¼-lb. cans, sealed, and stored at 25°C. The results (table IV) showed that there was a pronounced increase in total volatile base and trimethylamine in samples with a water content of between 11.6 and 15.5%. On the other hand total volatile base did not increase, and trimethylamine increased only very slightly in samples with a water content of 1.7 to 5.4%. The data obtained from bacterial counts in the few samples examined were inconsistent.

Table IV. Moisture, bacterial and volatile base content of dehydrated fish stored in sealed cans. A is value obtained on the freshly dehydrated product; B is value obtained after the storage period given.

Variety of fish	Time stored (days)	Water content		Viable bacteria (colonies per g.)		Total volatile base (mg. N per 100 g.)		Trimethylamine (mg. N per 100 g.)	
		A	B	A	B	A	B	A	B
Lingcod.....	97	1.7	1.8	4,700	11,000	27	27	4.2	8.9
Lingcod.....	118	15.4	15.5	930	*	24	84	2.6	40
Coho salmon....	118	11.6	12.0	4,700	410	25	55	3.2	13
Chum salmon...	105	4.1	5.4	23,000	1,300	38	35	0	2.6
Chum salmon...	118	12.3	12.2	1,800	2,000	37	69	4.2	19

*36,000 moulds per gram; these presumably prevented growth of bacteria.

SUMMARY

Precooked fish, dehydrated in heated, moving air, became contaminated with bacteria, moulds and yeasts during preparation.

In samples of dehydrated fish stored at 25°C. visible mould developed only at 80% or higher R.H., corresponding to a water content of 16.8 to 18.3%. Mould increased to an extent not visible to the naked eye in samples stored at 75% R.H., corresponding to a water content of 13.2 to 13.6%. The R.H.—water content relation of dehydrated fish was similar to that of precooked, air-dehydrated beef.

In two samples of dehydrated fish stored at 85% R.H., corresponding to a water content of 19 to 19.7%, the numbers of viable bacteria decreased sharply, while in a third sample there was a slight apparent increase. At higher humidities bacteria multiplied rapidly, while at lower humidities the viable bacterial population decreased in general more slowly in the drier than in the moister samples.

Both total volatile base and trimethylamine increased markedly in dehydrated fish stored at certain humidities at which viable bacteria and moulds decreased.

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REFERENCES

Bate-Smith, E. C., C. H. Lea and J. G. Sharp. *J. Soc. Chem. Ind.*, **62**, 100-104, 1943.

Cutting, C. L. *Chem. and Ind.*, **61**, 365-368, 1942.

Cutting, C. L., and G. A. Reay. *Chem. and Ind.*, **63**, 47-50, 1944.

Falk, K. G., E. M. Frankel and R. H. McKee. *J. Ind. Eng. Chem.*, **11**, 1036-1040, 1919.

Gane, R. *J. Soc. Chem. Ind.*, **62**, 139-140, 1943.

Henrici, A. T. Moulds, yeasts and actinomycetes, 1-296, Wiley, New York, 1930.

Macara, T. J. R. *J. Soc. Chem. Ind.*, **62**, 104-106, 1943.

Martinek, W. A., and B. Jacobs. *Fishery Market News*, **5**, 12-14, 1943.

Stevenson, C. H. *Bull. U.S. Fish. Comm.*, **1898** (18) 337-563, 1899.

Tarr, H. L. A. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **57**, 16-20, 1943.

Tarr, H. L. A., and B. E. Bailey. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.

Tressler, D. K. Marine products of commerce. 1-762, Chem. Catalog. Co., New York, 1923.

Young, L., and C. F. Lee. *Fishing Gaz.*, **60**, 64-65 and **74**, 1943.

Young, O. C., and E. P. Sidaway. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **56**, 7-9, 1943.

Variable Seaward Migration of Salmon

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ABSTRACT

Salmon (*Salmo salar*) kelts from hatchery operations, when liberated in rivers and in or near river estuaries, show by the distribution of the recaptured fish an extent of seaward migration that varies from river to river, apparently in relation to the character of the estuary and river influence in the sea. For the very large St. John river (N.B.) they are mainly in the long, deepish estuary and rarely leave the zone of river influence, while for the smallish Margaree river (N.S.), with a short, shallow estuary well emptied by the tide, they are mainly near the coast outside the estuary and commonly wander to distant places in various directions. This distribution may (Margaree) or may not (St. John) correspond with that of virgin fish. The Miramichi (N.B.) with strong river influence and long estuary resembles the St. John, while the Annapolis and Philip rivers (N.S.) with slight river influence are more like the Margaree.

For each river and location of liberation, the extent of seaward distribution is found to vary with differences in river discharge during a presumed settling-down period after liberation. Sharp freshets are correlated with more riverward distribution at least for a time. Heavy floods are correlated with a more seaward distribution. Continued low water is correlated with a wide distribution.

Do salmon travel back and forth between rather definite spawning grounds in the rivers and feeding grounds in the sea, or do they after spawning merely drop back to the sea a distance that varies with the conditions to which they are exposed?

Data are available for study of a possible relation between the extent of the seaward movement of the fish and the volume of the seaward flow of the river. Salmon from the operations of various Canadian hatcheries have been tagged and liberated by the Fish Culture service. The Director, Mr. J. A. Rôdd, has kindly furnished information supplementary to his published records of the tagging and recapture of these fish. The published records are in his Reports on Fish Culture for the various years, published separately and, starting with that for 1928, also in the Annual Reports of the Department of Fisheries of Canada. Records of liberations and recaptures up to 1923 are in his Report for that year, and later records appeared variously up to a final account of results in his Report for 1942. For river discharges there have been the published records of the Dominion Water and Power Bureau as given in its Water Resources Papers dealing with Atlantic drainage, published by the Department of the Interior and the Department of Mines and Resources of Canada from 1921 to 1942. These records have been supplemented by Mr. K. G. Chisholm, District Hydraulic Engineer.

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For the discharge from the estuary of the St. John river through the reversing falls, we are indebted to Mr. G. M. Brown of Saint John, N.B., District Engineer of the Department of Public Works, for providing records of the levels in the estuary. We have sometimes lacked discharge records and then have made use of rainfall records. The Meteorological Division of the Department of Transport has freely furnished its data.

As found in the early tagging of kelts in Scotland and in Norway (in Norway 3 per cent, Dahl and Sømme 1936) the percentage of fish recaptured in the Canadian experiments has been very small. The data for study of variable movement are, therefore, unavoidably few, and the conclusions therefrom clearly need further testing as data become available.

It is presumed that the salmon of any of the rivers considered do not differ in their tendency to move seaward. Also each river is considered separately, since there are such great differences between rivers in the seaward conditions. Since a relation was found between the extent of the seaward distribution and the distance seaward that the fish were liberated for the Restigouche river (Huntsman 1938), the rivers are primarily distinguished by the position of the point of liberation. However, the rivers differ in other ways that may be more significant for the extent of the seaward movement. To add to the complexity, strong flow sometimes carries the fish along and sometimes incites it to movement upstream, even in the case of a spent fish or kelt. It has been shown (Huntsman 1938) that sharp freshets, occurring with neap tides following liberation of spent salmon in the outer part of the Margaree estuary, are correlated with the occurrence of the tagged fish in the river above the head of tide the next spring.

The question to be asked for each type of liberation is—do the kelts there liberated move seaward a distance that varies with the character of the river discharge?

LIBERATION WELL UPRIVER

Salmon kelts were tagged and liberated from late October to early December about twelve miles (1 mile = 1.61 km.) above the head of tide in the Nictaux river, a tributary of the Annapolis river in western Nova Scotia. Recaptures, amounting to 4.5 per cent in the early years, were from "two principal areas *very* widely separated from each other,—the Nictaux river and the east coast of Newfoundland" (Huntsman 1938), as if the fish in part remained in the estuary (Annapolis basin) and in part went far in the ocean. Proof that some did not leave the estuary is not to be expected.

The estuary consists of the 25-mile long tidal part of the river, which gradually broadens, and of the Annapolis basin, with an area of about 35 square miles. The latter has full tidal interchange with the bay of Fundy through Digby gut, which opens abruptly on a straight coast, off which the water deepens rapidly, and along which play the strong currents of a 25-ft. (1 foot = 30½ cm.) tide. The maturing salmon are moving in the sea by the month of April and ascend the river early. On the outer coast they were taken by set nets at Gulliver Hole, which is situated about 9 miles seaward from the mouth of Digby gut and which would receive the ebbing outflow from Annapolis basin through the gut. No

tagged fish were reported from this fishery, which was declining during the period and ceased in 1940. Salmon are occasionally taken by the weirs in Digby basin and one of the tagged fish was reported taken therein as a kelt in the spring after liberation. Set nets are operated in the upper part of the estuary, and one tagged fish was reported as taken just below the head of tide in maturing condition the second spring after liberation. There is angling in the river in the spring, which gave most of the river recaptures. The ascending fish were trapped at Nictaux falls up to 1938, and the trap recaptured ten of the tagged fish.

The 40 recaptures from 895 fish show progressively more distant distribution during the two years following liberation. In the first year: April,—river; May,—river, estuary and Yarmouth shore; June,—St. John river outflow, Yarmouth shore and south Newfoundland; October to December,—east Newfoundland. In the second year: June, July,—Labrador coast. Times and distances involved are: 7 to 9 mos.,—50 to 700 miles; 12 to 14 mos.,—900 to 1,100 miles; 20 to 21 mos.,—1,300 to 1,900 miles. Recaptures of fish in the estuary or river, maturing for another spawning, were all made in May and June of the second year, that is after about 20 months, and these may not have left the estuary.

TABLE I. Percentage distribution of recaptures of tagged Annapolis salmon and mean discharge for half-monthly periods in Paradise brook and East Bear river. Cubic foot = 28.32 lit.

	Annapolis river				Paradise brook					East Bear river				
	Salmon tagged	Percentage recaptured			Discharge in cu. ft. per sec.					Discharge in cu. ft. per sec.				
		Distant	River	Total	Oct.	November		December		Oct.	November		December	
1933	140	0	6.4	6.4	319	228	195	148	142	564	396	373	267	206
1931	261	1.5	1.9	3.4	31	72	58	83	106	43	99	77	155	194
1932	165	3.0	1.8	4.8	55	84	207	90	78	123	186	202	218	157
1936	92	2.2	2.2	4.4	108	151	117	207	156	246	200	267	497	420
1934	76	2.6	0	2.6	41	198	125	380	93	58	402	223	592	238
1937	124	2.4	0	2.4	76	72	114	112	82	181	180	282	285	285
1938	37	2.7	0	2.7	65	54	121	350	115	91	99	203	459	227

There was considerable variation between those tagged in the different years as to the seaward distribution of the recaptures (table I). Those liberated in the years 1931, 1932 and 1936 gave distant recaptures as well as river recaptures in the second year, which may mean that some remained in the estuary while others went far oceanward and northward. The percentages recaptured were 3.4, 4.8 and 4.4 respectively. Those liberated in 1933 had recaptures only in the river and in the second year, as if none had left the estuary, and the percentage recaptured was high, 6.4. Those liberated in 1934, 1937 and 1938 had no recaptures in the river in the second year, as if all had left the estuary, but, with the trap not operating, recapture in the river of the fish liberated in 1937 and 1938 could only be by the set nets and the rods, which had taken only about half of the tagged fish recaptured in the river. The percentages recaptured were

low, 2.6, 2.4 and 2.7 respectively. This seems to mean that the smaller the proportion that remained in the estuary and returned to the river, the smaller the percentage of recaptures, that is that they were less apt to be caught out in the ocean, which is perhaps to be expected.

Records of discharge are not available for the Nictaux and Annapolis rivers, but they are available for Paradise brook and the east branch of Bear river, which empty into the same estuary, the former at the head of tide adjacent to the Annapolis and Nictaux. It will be seen in table I that for the year 1933, the recaptures of whose fish were most numerous and all in the river, the mean discharge in each half month for both streams dropped steadily from October to December, while for 1938, with few recaptures and these all distant, the mean discharge increased strikingly from early November, when the fish were mostly released, to early December. In 1934, with few and all distant recaptures, freshets came just after the end of tagging (November 10), and also in early December. In 1937, also with few and all distant recaptures, moderate freshets came as the fish were released after the middle of November and also in early December. In the years 1931, 1932 and 1936, with both distant and river recaptures and an intermediate total percentage of recaptures, river discharges showed an intermediate condition, neither declining steadily nor showing freshets both after tagging and in early December. There is some evidence, therefore, that freshets after tagging and before the fish were settled down for the winter determined the extent to which the fish were carried seaward.

LIBERATION ABOVE HEAD OF TIDE

Kelts were tagged and liberated in November and early December in Philip river, northern Nova Scotia, at the upper dam near Oxford Junction, which is not far above the head of tide. Recaptures, amounting in the early years to only 1.3 per cent, were (Huntsman 1938): (1) as kelts in the river until the following spring, (2) as ripe fish in the river in the second autumn, and (3) in distant places (south and east coasts of Newfoundland).

The estuary is 13 miles long, shallow and narrow, and it discharges across a bar into Pugwash bay with extensive shallows and the slight tides of Northumberland strait. The maturing salmon seem not to be moving in the sea before June or July and do not enter the river before August or September. Netting them in the outer estuary has been abandoned, and was apparently never successful on the coast. Poachers take them with nets in the upper estuary. In the river, angling is successful only for the kelts in the spring, and the maturing salmon are taken in the trap which takes part of the run from September on.

About 500 fish were tagged in each of the years 1938, 1939 and 1940, from which there were 57 recaptures, making 3.8 per cent. The percentages recaptured in the three ways differed considerably for the different years, as shown below.

	Kelts in river	Ripe fish in river	Distant	Total
1938	0.2	0.4	0.2	0.8
1939	0.2	2.3	1.4	3.9
1940	1.8	4.2	0.8	6.8

One of the ripe fish in the river for the 1940 fish was taken the first autumn after tagging, and also one of the distant recaptures was still a kelt when taken the following June near Arisaig, Antigonish county, N.S. Other distant recaptures were for the first year after tagging at Antigonish, Margaree coast (Cape Breton) and Fishing Ships harbour (Labrador), and for the second year Little harbour in Pictou co., Morristown, George bay, Monk head and Pomquet in Antigonish co.; Chedabucto bay in Guysboro co.; and Tors cove and Trinity bay in Newfoundland. Only 1939 fish were recaptured at Labrador and Newfoundland. Fish of all three years were recaptured on the northern coast of Nova Scotia.

No discharge records for the river or for any neighbouring river are available. The low returns everywhere for the 1938 fish give no indication as to where the fish went. That they should be almost wholly lost to the river might be expected from the fact that C. Sayer, who was in charge of the handling of the fish reported "unusually heavy freshets while the fish were impounded. At one time it was necessary to seine up all the salmon and place them in pontoons attached by cable to the shore until the water level dropped." The fish were impounded by November 1, and were tagged and liberated from November 8 to 14. Spring tides reached their maximum for the month on November 11. In 1940, which had a high percentage of returns, nearly all local, I. A. Mowat, who was in charge, reported high water hampering the work at the beginning of the season (September 23), but that the run of fish (the best ever observed in the cultural operations there) continued to December 1 at least, although none were taken after November 16. The fish were tagged from December 6 to December 14. The maximum spring tide was on December 1. While no heavy flood like that of 1938 was reported, there were doubtless fairly good and frequent freshets as judged from (1) the unusually good and late run of salmon, and (2) the rainfall at Nappan (November—4.83 and December—4.13 inches). Frequent freshets, as compared with a heavy flood, seem to have held the fish in the river, which effect is comparable with sharp freshets and neap tides following liberation of kelts in the Margaree estuary taking them up the river (Huntsman 1938). The contrasted condition of practically no freshets occurred in 1939, which had little more than half as many recaptures, and a high proportion of them distant, including some very distant ones. C. Sayer reported "low water throughout the whole season" and the rainfall at Nappan was 1.23 and 3.08 inches in November and December.

There is, therefore, evidence that the seaward distribution of the salmon liberated above the head of tide in the Philip river is affected by the character of the river discharge following liberation.

LIBERATION IN INNER ESTUARY

Kelts were tagged and liberated in the Northwest Miramichi branch of the Miramichi estuary over 35 miles inside the estuary mouth at Portage island, on the east coast of New Brunswick in the autumn in various years. Recaptures, amounting to 4.1 per cent., were nearly all in the estuary or above and mainly in the inner estuary, with a very few far distant (Huntsman 1938).

The estuary consists of an inner forked part (Northwest and Southwest), each fork about 15 miles long, a middle narrow part about 15 miles long, and an outer broad part (inner Miramichi bay) also about 15 miles long. Outside the outer islands, the bay broadens out into the gulf of St. Lawrence. The estuary is quite shallow and has several connections with the bay, whose depth increases very gradually going offshore. The maturing salmon begin to move about near the surface in May and run chiefly in June. They are taken extensively in drift nets offshore in the gulf and in set nets in the estuary, as well as by angling up the rivers. The set nets take them in the inner estuary (Northwest) in the fall for hatchery purposes. They are also taken by angling in the branches of the river above tidal water.

It is a striking fact that from 2,104 kelts liberated from 1913 to 1928, of which 87 recaptures were reported, not one was reported from the outer waters (outside Pointe du Quart) where the bulk of the commercial catch of salmon is made, e.g. 88 per cent in 1926 and 97½ per cent in 1930, the years in which returns from the last two liberations would be expected. The obvious conclusion is that the liberated fish were practically restricted in their distribution to the estuary inside Pointe du Quart. It would seem that, under these circumstances, little effect of variable river discharge on the seaward distribution of the fish could be apparent, only two fish having been reported from distant places. However, data of distribution of recaptures are given in table II for years for which discharge records of the Southwest Miramichi river are available. It may be presumed that discharge for the Northwest Miramichi river would not differ greatly from that of the other branch. The data are arranged in order of the amount of the discharge. It will be seen that the distant recaptures were only with the highest and lowest discharges, and that on the whole with intermediate discharges the recaptures are high in number and as kelts in the estuary in the spring. This corresponds with the conception that with heavy floods and drought the fish are spread widely, but with sharp freshets they largely stick to the estuary and river.

TABLE II. Distribution in time and space of recaptures (percentages) of salmon tagged and liberated as kelts in the Northwest Miramichi estuary in various years, together with the mean discharge of the Southwest Miramichi river for a fifteen-day period subsequent to tagging (Nov. 6-20).

Year	1924	1921	1922	1928	1923	1917	1918	1919	1920
Number tagged	195	161	149	100	150	149	149	100	188
Mean discharge (cu. ft. per sec.)	655	1,471	1,730	2,295	2,610	High	3,541	3,980	4,062
Kelts in spring (%)	4.6	5.5	4.7	7.0	4.7	0	0	0	3.2
Clean fish in 1st summer. (%)	0.5	0	0	0	0	0	0	0	0
Clean fish in 2nd year in river. (%)	0.5	0	2.7	0	0	0.7	2.7	0	0.5
Distant	0.5	0	0	0	0	0	0	0	0.5

In 1937 from October 27 to November 9, 608 kelts were tagged and liberated and a variable seaward distribution is apparent in relation to time of liberation. This variable distribution may be due to river discharge. No records of the discharge are available, but the precipitation records for successive five-day periods, as taken at Chatham and Burnt Church on the estuary and at Hardwood Ridge, not far from the most southerly head waters of the river, show the following average values in inches: October 27-31, 1.35; November 1-5, 0.08; November 6-10, 0.65; November 11-15, 2.22; and November 16-20, 1.44. The fish liberated later were evidently subjected to heavy floods while settling down. Whatever may be the explanation, the recaptures in the middle and outer estuary

TABLE III. Recaptures (percentages) in various waters seaward, of salmon tagged as kelts in the inner Miramichi estuary in 1937. Those liberated earliest (October 27 to 30), totalling 182, are shown in the percentages by figures in italic type (*2*); those liberated from November 1 to 5, totalling 241, in roman type (2); and those liberated latest (November 6 to 9) totalling 185, in heavy type (**2**).

	Inner estuary and above			Middle estuary			Outer estuary			Outside waters			Newfoundland		
December (kelts)					0.4	**0.5**			**0.5**						
First spring (kelts)	*5.0*	5.8	**2.2**	*1.6*	2.9		*1.6*		**0.5**						
First summer and fall	*0.5*			*0.5*										0.4	
Second summer and fall	*0.5*	0.8	**0.5**	*0.5*	1.2						0.4	**1.1**			**0.5**
Third spring (kelts)	*0.5*				0.4										

in December of that year showed only the later fish, and the latest were farthest out and most numerous (table III). Corresponding with the conclusion that the latest fish went farthest seaward, these fish were least numerous in the river and estuary both the next spring as kelts and later, and they gave most recaptures in outside and distant waters. It may be noted that, in contrast, the earliest liberated were unrepresented in outside and distant waters, and were the only ones recaptured in the river estuary the first summer and fall after tagging. Together, these facts give rather strong evidence of a differential seaward distribution related to time of liberation.

LIBERATION IN OUTER ESTUARY

Kelts were tagged and liberated about a mile inside the mouth of the 5-mile long Margaree estuary on the west coast of Cape Breton island in the autumn in various years. Recaptures were reported up the river, in the estuary, along both west and northeast coasts of the island, along the mainland of Nova Scotia to the south from Pictou to Chedabucto bay, all around Newfoundland, and along the coast of Labrador (Huntsman 1938).

The estuary is very shallow and has a narrow mouth which has been deepened so that it fills and empties rather completely with the tide. As a result, fish tend to be swept out of it with springing tides (Huntsman 1938, p. 113). The estuary opens on a rather straight coast, along which there is a pronounced set to the northeast out of the gulf, superimposed upon tidal ebb and flow. The maturing salmon start to move about near the surface in early June, beginning to enter

the river at that time, but entering chiefly with high water in the fall. They are taken by angling in the river and by set nets or traps all along the coast. A trap in the estuary takes them in late summer and fall for hatchery purposes.

In strong contrast to the Miramichi, the Margaree salmon tagged as kelts have been recaptured mainly where the bulk of the commercial catch is made, that is, along the 20-mile stretch of coast adjacent to the mouth of the estuary. Also, these are taken largely in the second summer after liberation. Associated with this contrast is the rather large proportion of fish distributed rather far to the south and west and very far to the north and northeast. This may be correlated with the absence of conditions suitable for them to remain in the estuary.

In 1916, with sharp freshets rather than heavy floods, 11 out of 114 fish liberated with neap tides on November 15 and December 1 were recaptured still as kelts in the river the next spring, and 4 were taken the second year after in the sea. As a contrast, in 1917 not one of 99 fish liberated on November 20 and 22 was recaptured during the ensuing year, but 7 were taken in the second year, 6 in the sea and 1 in the river, while a heavy flood of 5,750 cu. ft. per sec. occurred on November 24 (Smith 1925) and springing tides reached their greatest amplitude on November 28.

Like 1917, in 1922, 103 fish liberated on November 15 and 16 with spring tides of greatest amplitude on November 18 and with high water from November 3 to 12 and 19 to 30 gave only two recaptures, both in the second year. Of 99 fish liberated in 1923, with a heavy freshet from November 23 to December 1, reaching as much as 3,300 cu. ft. per sec., and with spring tides of greatest amplitude on November 25, those liberated on November 24 gave two recaptures in the second year, while those of December 4 and 5 gave two in the river the ensuing spring. Of 99 fish liberated on November 21, 1924, one was recaptured in the river as a kelt in the ensuing year, and seven were taken in the second year; there was a moderate freshet (1,410 cu. ft. per sec.) on November 24 and spring tides had greatest amplitude on November 29.

In 1934, there was a very heavy flood from November 5 to 8, reaching a discharge of 5,530 cu. ft. per sec., and there were later freshets of more than 1,000 cu. ft. per sec. on November 11-12, November 20, and November 30-December 2. The number of fish released on each day and the distribution in time of their recaptures, were as follows:

	November						December		
	13	15	19	21	26	28	3	5	6
Number	45	57	52	60	48	45	56	144	29
First year as kelts	0	0	0	0	2	2	2	0	0
Second year	2	8	3	2	3	1	3	1	1

The floods were perhaps acting on all the lots of fish, but no very heavy flood coincided with springing tides. The poorest tides were from November 28 to December 3 and seemingly related to these were the recaptures as kelts of fish liberated around that time.

In 1936, there were freshets of over 1,000 cu. ft. per sec. on November 23-24, 26-27, December 4 and 7-8, and there was a heavy flood from December 11 to 15,

reaching 5,230 cu. ft. per sec. The number of fish released each day and the distribution in time of their recaptures were as follows:

	November	December		
	25	1	5	10
Number	53	53	40	97
First year as kelts	3	4	1	1
Second year	4	3	0	1

The heavy flood occurred with springing tides reaching their maximum on December 15, after all the fish had been liberated. It seems to have affected the fish more recently liberated, and it may be of significance that those recaptured as kelts from the successive lots were taken as follows: On the Margaree coast for November 25; in the river and on the Margaree coast for December 1; at Cap Rouge about 25 miles with the current for December 5; and off Port-aux-Basques, Newfoundland, over 100 miles with the current for December 10. The last was reported as clean rather than a kelt, but it was taken in May.

Floods with springing tides seem to have swept out to sea kelts that had not become firmly established.

LIBERATION OUTSIDE ESTUARY

Kelts were tagged and liberated in Courtenay bay, just outside and to the east of the mouth of the estuary of the St. John river at Saint John, in the autumn in various years. Of the 2,021 fish liberated from 1913 to 1930, recaptures, amounting to 2.3 per cent, were reported only from the river, the estuary and the outflow of the same in the bay of Fundy (Huntsman 1938).

The estuary is 80 miles long, with branches, and a fair amount of rather deep water. It is so narrow and shallow at the reversing falls inside St. John harbour that all of it inside this point shows very slight tidal effects, while the waters of the harbour and bay have a great tidal amplitude. The maturing salmon begin to move about near the surface in both the bay and the estuary in late May and migrate inwards. They are taken by drift nets in the outflow in the harbour and bay, by weirs in the harbour, by set nets or traps in the estuary and by angling up the river. They were formerly also taken by set nets up the main river.

Similarly to what was found for the Miramichi, the recaptures from 2,021 fish liberated up to 1930 were almost wholly in the estuary and river (44 out of 46), that is, inward from the point of liberation, whereas the bulk of the commercial catch is made outside (Huntsman 1938). It is clear that most of the fish move inwards shortly after liberation, some being recaptured 40 miles inward in a month and a half after liberation, and that there is very little movement seaward.

A large lot (657) of kelts tagged and liberated in 1938, showed in recaptures a striking difference in distribution from the earlier lots, and may be compared (fig. 1) with a lot of 806 kelts liberated in 1930. As shown in the table below, in percentages of recaptures for the two lots, the "1930" fish were taken mainly in the river, far inward from the point of liberation; the "1938" fish were taken

	River	Estuary	Harbour	Bay	Newfoundland
1930.....	1.49	0.24	0.37	0.12	0
1938.....	0.15	0.30	0.91	1.98	0.15

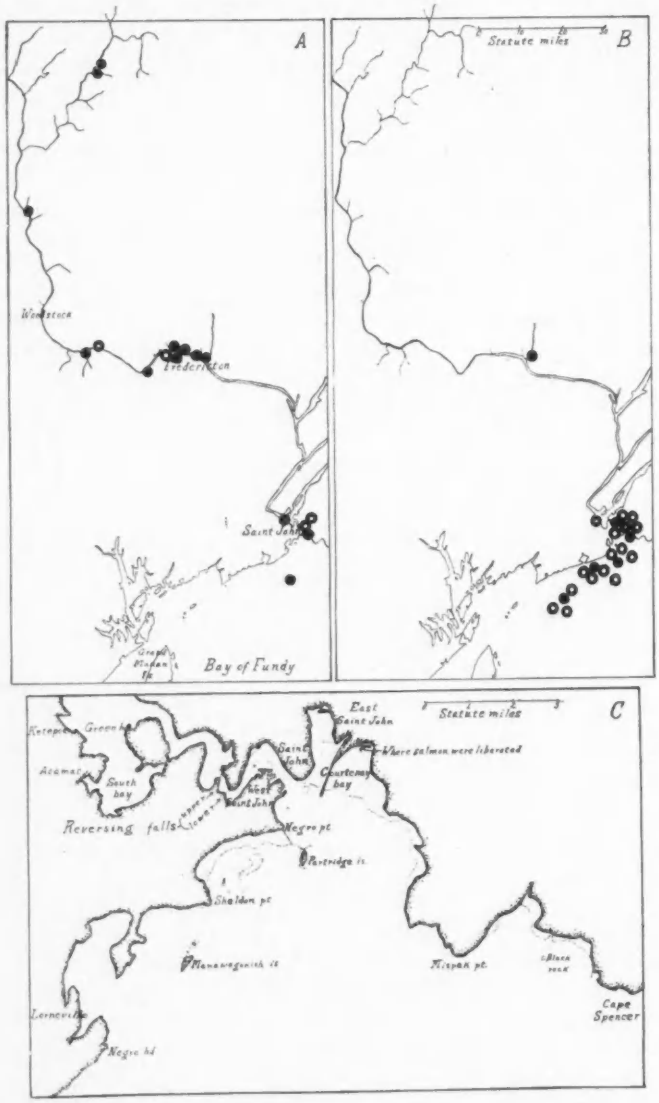

FIGURE 1. A and B: Recaptures in St. John river and estuary and neighbouring bay of Fundy, of salmon tagged and liberated as kelts in 1930 and 1938 respectively. Circles with clear centres represent salmon recaptured in first year and solid circles in second year after liberation. C: Mouth of estuary, showing positions of Reversing falls and of Courtenay bay, where the salmon were liberated at high tide (dotted line is low tide mark).

mainly in the bay, outside the point of liberation. Also, a much larger proportion (3.5 vs. 2.2%) of the "1938" fish were recaptured than of the "1930" fish, and while the former were taken mainly in the first year, (2.7 vs. 0.8%), the latter were taken mainly in the second year (1.6 vs. 0.6%). The fish in the bay are subjected to very intense fishing near the estuary mouth and may mature earlier through having an exceptional abundance of food.

That the "1938" fish were mainly out in the bay, as the above facts show, is clearly the reason for one being recaptured in a distant place, the first record of

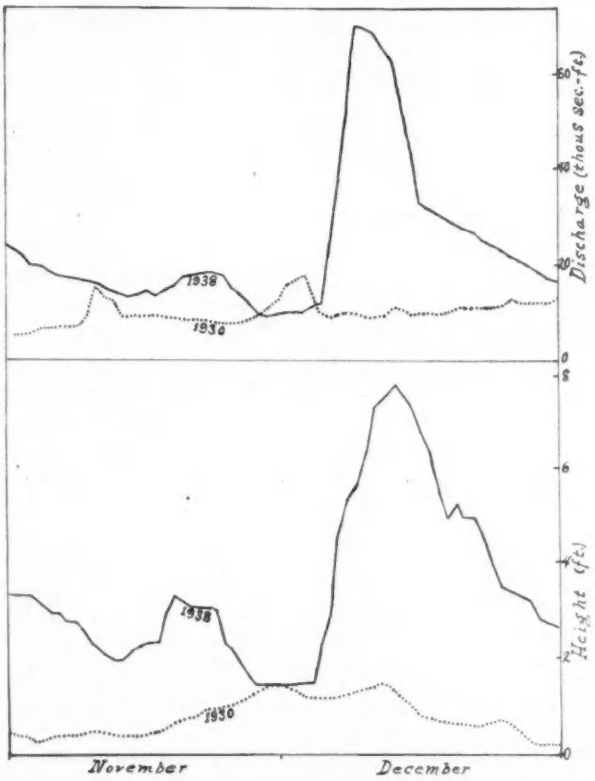

FIGURE. 2. Discharge of St. John river at Pokiok (upper) and height of St. John estuary at Oak point (lower) in November and December of 1930 and 1938, from the official records.

such. Like the Annapolis fish of the other side of the bay of Fundy, this fish was taken on the east coast of Newfoundland in the late autumn of the yea after liberation, which has not occurred for salmon from any other rivers.

It is a striking fact that 5 of the 12 "1930" fish taken in their second year were at or above the head of tide in May of that year, and that 3 more were, one in early June, well up towards Woodstock, and two far up the Tobique in late June. This behaviour is that of the late autumn virgin fish that enter the

estuary from November to January, the period during which the tagged kelts also definitely entered. The fish tagged as kelts, however, ascended the second spring after entering, not the first spring as do the virgin "winter" fish. The "1938" fish taken in their second year were, with the exception of one in the Nashwaak river in April and of doubtful significance, all taken outside the estuary, and doubtless did not ascend the river until late in the season, showing behaviour very different from that of most of the "1930" fish.

Since there is some evidence that the tagged fish, liberated early in November, enter the estuary with the late autumn virgin fish before winter sets in, several having been taken in December 40 miles up the estuary, the question arises as to whether the conditions for their entrance were in any way different in 1938 from what they were in 1930. Fishermen who drift for salmon in the harbour, that is in the estuary mouth outside the reversing falls, state that they get most salmon when the river is in heavy flood, but, when the freshet subsides, they no longer get them, since they go quickly past the reversing falls into the estuary. Data for the river discharge at Pokiok above Fredericton, furnished by Mr. K. G. Chisholm, District Hydraulic Engineer, and for the height of the lower part of the estuary at Oak point, furnished by Mr. G. M. Brown, District Engineer of the Department of Public Works, have been plotted in figure 2 and are similar to each other. They show that the lower estuary, which determines outflow through the harbour, was in 1938 never lower in November and December than its highest level in 1930, with very high water during most of December. The records available for other years (1918 to 1934) reveal nothing at all comparrable to this December freshet. On the basis of the experience of the fishermen, such a freshet might well have kept the kelts from entering before the winter set in, which would explain the extended outward distribution of the "1938" fish.

DISCUSSION

The usual seaward distribution of the salmon liberated as kelts clearly varies from river to river, perhaps chiefly in relation to estuarial conditions. Large rivers with strong influence and with long and not too shallow estuaries retain most of the fish well in the estuary, as do the large Miramichi and very large St. John rivers. The much smaller Annapolis, with the outer estuary deep and with free tidal interchange through a break in a straight coast swept by strong currents, seems to lose about half its fish, which wander very widely out of the bay and to the northeast. The Margaree, of similar size, with a very shallow estuary with free tidal interchange through a break in a somewhat straight coast along which moderate currents pass, apparently has all its fish leave the estuary and river by the summer following liberation, most remaining within twenty miles of the estuary mouth, but a substantial portion wandering widely in both directions coastwise and far to the northeast. The Philip river is smaller than and apparently similar to the Margaree. Such seaward distribution may for some rivers differ greatly from that of the virgin fish (those that have gone seaward only as smolts); at least the bulk of the virgin fish of the Miramichi and St. John rivers are much farther seaward, which is not true for the Margaree.

It is worth notice that salmon that have spawned and are presumably ready

to move seaward should move riverward into and through the 80-mile long St. John estuary and up the river proper at least 10 miles (Huntsman 1938). It is remarkable that such spawned fish should remain in the estuary, as the facts of recapture indicate that most of them do, until they ascend the river for second spawning, although they evidently grew from smolts to first sexual maturity out in the bay, as shown by their being first captured in the harbour as maturing fish, as well as by the fishery showing that the virgin fish are out in the bay at first. While all or practically all the kelts clearly leave the Margaree estuary for the sea, some of those liberated in the outer estuary at first move riverward and ascend at least as far as fourteen miles above the head of tide (Huntsman 1938). In 1935 a kelt was observed regularly until late in July in the Northeast Margaree river about six miles above the head of tide. It usually occupied a definite lie under a log projecting into the current. In 1935 and again in 1937, large, bright, slink salmon that had spawned were observed to enter the cool Trout brook near the head of lake Ainslie (the source of the Southwest Margaree river) in late summer when the lake water became very warm. No evidence was found that the lake contained salmon that had failed to go to sea between the smolt stage and first spawning. These facts should be coupled with those showing that some salmon tagged as kelts may be nearly five hundred miles away from their river, e.g. Margaree fish on outer Labrador coast, in less time than what others spend far inward from the point of liberation. The great variety in seaward-migrating behaviour is a striking fact.

With such great variation in extent of seaward migration, it should be reasonable to expect that most of the fish would show neither extreme, but an intermediate type of behaviour. The facts indicate that most of the St. John fish entered and remained in the estuary, except in 1938. Also they indicate that most of the Margaree fish left the estuary and remained in the vicinity of the Margaree coast. Migration of some of the fish to a great distance in the sea, as to Newfoundland, seems to be a regular occurrence for the Margaree, but decidedly unusual for the St. John.

Movement of tagged kelts up river from the estuary has been correlated with sharp freshets or rapidly lowering discharge for the Margaree river (Huntsman 1938). Similar correlation of upward movement of virgin maturing salmon with sharp freshets was the basis for experiments on the movement of these under experimental conditions with artificial freshets. Sharp freshets were found to be very effective (Huntsman 1943), even with very low and warm water, which has been considered unfavourable for salmon ascent. Fish liberated far up river in the Nictaux branch of the Annapolis river could not be expected to show this effect, but those liberated in 1933 with lowering discharge and slight freshets showed least tendency to leave the river. Similarly kelts liberated in the lower part of the Philip river in 1940 with exceptionally good conditions up to December for fresh-running salmon showed least tendency to leave the river. The same holds for the Miramichi river in 1923 and 1928. Data are not available to show whether sharp freshets affect upriver movement of the St. John kelts.

Heavy floods have a contrary effect, carrying the kelts seaward. That seems indicated for the Annapolis river in 1934 and 1938, for the Philip river in 1938, for the Miramichi river from 1917 to 1920 and late in 1937, and for the Margaree

river when coupled with spring tides in various years, 1917, 1922, 1923, 1924, 1934 and 1936. For the St. John river, a heavy flood in 1938 clearly kept most of the tagged kelts from entering the estuary and distributed them well out in the bay. It has been well established that a rise of water is an important factor in smolt descent (Barry 1932 and 1933, White 1939) and kelts might be expected to be similarly affected by a big rise in water level. Observations made on the Margaree river in 1935, and subsequently, showed that the parr are carried downstream by floods and the estuary and its tributary streams populated with young salmon. Also observations showed that virgin maturing salmon are at least held back in their ascent of the river by heavy floods.

A third condition, lack of freshets, seems to give the most general distribution of the fish. For the Annapolis river, continuing low water in 1931 was associated with recapture of the tagged fish not only in the river, but in the estuary and at Newfoundland. For the Philip river in 1939, with "low water throughout the whole season", there were recaptures in the river, on the Antigonish coast, in Chedabucto bay, on the Margaree coast, on the Newfoundland coast and on the Labrador coast. The Miramichi salmon liberated in 1924, with no very high water and with slow changes in volume of discharge, gave recaptures not only in the inner estuary, but in the outer estuary and one on the east coast of Newfoundland, an exceptional record. This is in agreement with what seems to be a general tendency for salmon—to wander when away from a directive river influence, as well as to leave shallow water. Low water in the river means not only shallowness, but slight river influence in the sea.

The possibility of a general effect of tide at time of liberation on the distribution of the fish has been explored without success, as also the possibility of the survival of the fish being affected by their being swept suddenly out to sea.

The principal distribution of the fish is along the river and its outflow in the sea. Rarely do the St. John salmon go beyond the evident river influence in the sea, while it is quite usual for some of the Margaree fish to do so. The other rivers seem to vary in this respect between these two extremes. The direction of the distant movement varies, but it is largely to the northeast and as far as 1,900 miles for an Annapolis fish taken in northern Labrador. This direction is that prevailing for the surface water in summer. Similarly, kelts tagged in Scottish rivers were recaptured at a great distance mainly to the south on the east coast (Menzies 1925, p. 46), the direction of circulation of the water as shown by drift bottles (Helland-Hansen 1912, p. 262).

As in all tagging experiments, it is an open question whether these tagged kelts behave the same as do untagged kelts. No method of settling the matter is apparent, but, where comparisons have been possible, we have as yet failed to find any difference in behaviour. Nevertheless, the results are for fish kept in confinement and released as kelts at the times and places given, and we cannot state how applicable they are to salmon under natural conditions.

REFERENCES

BERRY, J. Report of an investigation of the migration of smolts in the river Tay during spring 1931. *Fish. Scot., Salmon Fish.* **1931**, VI, 1-22, 1932.

Notes on the migration of salmon smolts from Loch Ness; summer 1932. *Fish., Scot., Salmon Fish.*, **1933**, I, 1-12, 1933.

DAHL, KNUT, and SVEN SOMME. Experiments in salmon marking in Norway 1935. *Skr. Norsk. Videns.-Akad. Oslo, Mat.-Nat. Kl.* **1935**, 1-27, 1936.

HELLAND-HANSEN, B. Physical oceanography. In J. Murray and J. Hjort, The depths of the ocean, 210-306, London, 1912.

HUNTSMAN, A. G. Sea movements of Canadian Atlantic salmon kelts. *J. Fish. Res. Bd. Can.*, **4**, 96-135, 1938.

Atlantic salmon and trout investigations. *Ann. Rep. Fish. Res. Bd. Can.*, **1942**, 29-30, 1943.

MENZIES, W. J. M. The salmon, its life history, i-xii, 1-211. Edinburgh and London, 1925.

WHITE, H. C. Factors influencing descent of Atlantic salmon smolts. *J. Fish. Res. Bd. Can.*, **4**, 323-326, 1929.

Glycerol Yield from Dogfish Liver Oil

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ABSTRACT

Variations from 8.15 to 0.96 per cent in the glycerol yield from twelve dogfish (*Squalus suckleyi*) liver oils are reported and shown to be inversely related to their 7.6 to 34.3 per cent yield of unsaponifiable matter, or, better, to their 6 to 31 per cent yield of glyceryl ethers. The glycerol determinations are confirmed by an independent method involving a comparison of the hydroxyl group and replaceable hydrogen contents of the hydrolyzed oils.

Fatty oils show wide differences in their composition depending upon their source, so that the determination of certain characteristics frequently enables their origin to be stated with fair accuracy. Some fish liver oils contain almost no unsaponifiable matter ("unsap"). Such an oil consists usually of a considerable variety of fatty acids combined in triglycerides, from which is liberated by hydrolysis an amount of glycerol equal to approximately 10 per cent of the weight of the oil. For example, a sample of herring oil containing only 1.4 per cent "unsap" yielded 10.3 per cent glycerol on saponification, determined by the method described below. At the other extreme of variation in composition, Tsujimoto (1916) reported a shark liver oil, later identified as from *Centrophorus* sp. (Tsujimoto 1920), which yielded 90.2 per cent "unsap" and only 0.52 per cent glycerol; 85 per cent of the oil consisted of a hydrocarbon which he identified as squalene.

Oils from individual fish of any one species do not show such wide differences in composition as indicated above, but the proportions of the characteristic components of an oil may vary from fish to fish in the same species. The yield of "unsap" from pilchard oil is between 0.1 and 1.23 per cent, a twelve-fold variation (Brocklesby *et al.* 1941, p. 385). The liver oil of the Pacific dogfish (*Squalus suckleyi*) is reported to yield from 5 to 25 per cent "unsap" (Brocklesby *et al.* 1941, p. 407). In the course of an investigation on the composition of the "unsap" of the liver oil from individuals of this latter species, an oil (PR 3) was examined of which the "unsap" yield was 34.3 per cent, greatly exceeding the range previously reported. This oil yielded 0.96 per cent glycerol.

The "unsap" of dogfish liver oil has been shown both elsewhere (Toyama and Tsuchiya 1927, J. D. Cawley unpub. 1942) and in this laboratory (unpub.) to consist largely of glyceryl ethers, mainly selachyl alcohol with some chimyl alcohol and small amounts of highly unsaturated ethers (Toyama and Takahasi 1939). The ethers so far reported may all be thought of as consisting of a mole-

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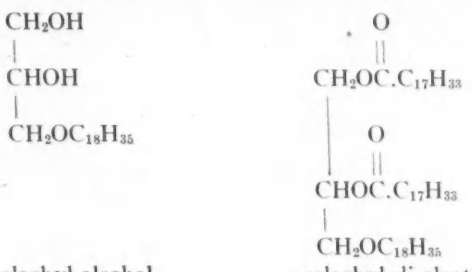
cule of glycerol attached through an ether linkage at one of its α-carbons to a long straight-chain alkyl group. The two hydroxyl groups remaining are normal-



selachyl alcohol — selachyl di-oleate

ly esterified, as observed by André and Bloch (1932) in the liver oil of *Scymnorhinus lichia*. Low hydroxyl values of oils from different dogfish livers were obtained by the writers, indicating that the glyceryl ethers in this liver oil also are almost completely esterified (table II).

If selachyl alcohol (mol. wt. 342) and selachyl di-oleate (mol. wt. 870) are considered, it is seen that the ester yields 39.3 per cent "unsap" on saponification. Or, in other words, 1 g. of the free ether-alcohol produces 2.54 g. of oleic ester. Now if a fat is imagined with the following characteristics:

—free hydroxyl value and free fatty acid value are both zero,

—oleic acid is the only fatty acid present,

—"unsap" yield is 20 per cent of the fat and the "unsap" contains only selachyl alcohol,

then this fat must contain 50.8 per cent selachyl di-oleate (20 × 2.54) and therefore only 49.2 per cent glyceryl tri-oleate. The glycerol obtainable from this fat can be only about one half that (10.4 per cent) from the same weight of pure glyceryl tri-oleate.

Actually, the relationships are not so simple since every natural fat contains a wide variety of fatty acids and a variety of substances in its "unsap". For example, in the liver oil from *S. lichia* mentioned above there was 64 per cent "unsap" which was about 89 per cent hydrocarbon, corresponding to 57 per cent hydrocarbon in the oil; the oil was demonstrated to be 21 per cent glyceryl ether esters. This leaves 22 per cent (100 − 57 − 21) as the maximum possible content of glycerides, corresponding to approximately 2.2 per cent yield of glycerol from the oil. André and Bloch found 1.34 per cent glycerol.

These calculations could not be expected to hold exactly for any natural fat. However, applied to dogfish liver oil PR3 they yield the results shown in figure 1 in which the composition of 100 g. of oil is pictured. Line 1 indicates that it consists of non-esterifiable "unsap", esterified "unsap", and glycerides. Components of the non-esterifiable "unsap", for example hydrocarbons, are in the same uncombined condition both in the oil and in the extracted "unsap". Line 2 shows the products of hydrolysis of the oil. Line 3 shows the approximate content of glyceryl ethers in the "unsap" as determined by chromatographic adsorption (the method to be described in a later communication). The remaining "unsap" contained 1.27 g. cholesterol (3.7 per cent of the "unsap" by the

Liebermann-Burchard reaction) and 0.64 g. vitamin A. (The oil had a potency of 27,400 U.S.P. units per gram, and there are 4,300,000 units in 1 gram of crystalline vitamin A (Hickman 1943, p. 354).) This leaves of undetermined composition 1.5 g. of the "unsap" (3.4 − 1.27 − 0.64). Line 4 gives an estimate of the weight of esterified glyceryl ethers in the oil, based on the assumption that they are selachyl alcohol esterified with oleic acid. This assumption is known to be actually only partly correct, as mentioned above. In the remaining

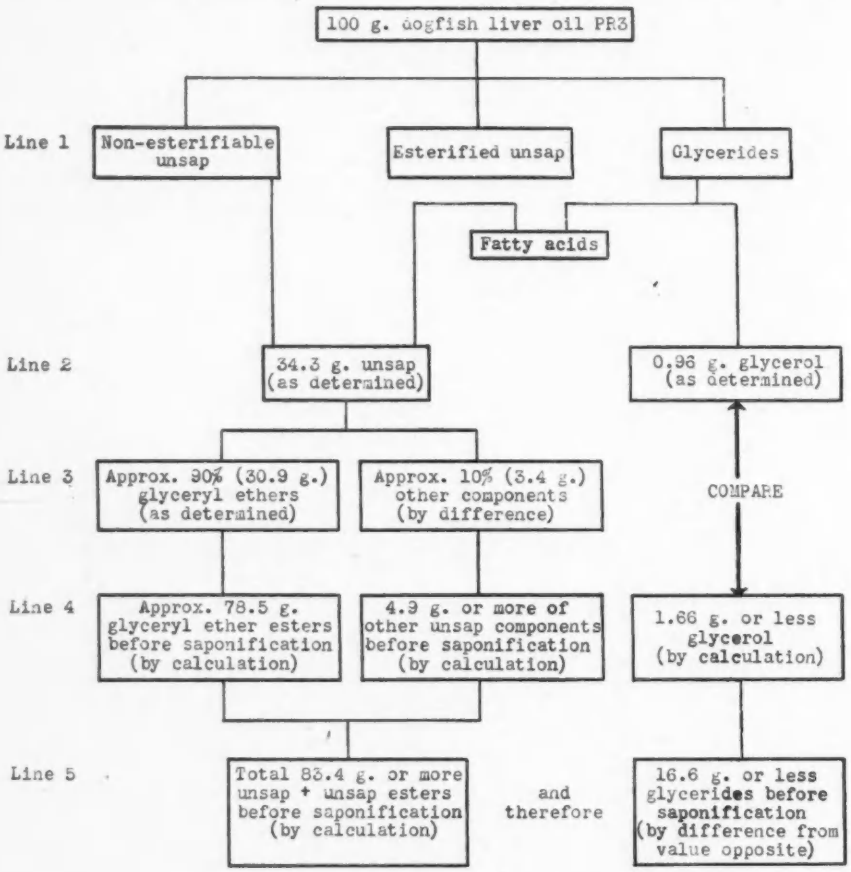

100 g. dogfish liver oil PR3
Line 1
Non-esterifiable unsap
Esterified unsap
Glycerides
Fatty acids
Line 2
34.3 g. unsap (as determined)
0.96 g. glycerol (as determined)
Line 3
Approx. 90% (30.9 g.) glyceryl ethers (as determined)
Approx. 10% (3.4 g.) other components (by difference)
COMPARE
Line 4
Approx. 78.5 g. glyceryl ether esters before saponification (by calculation)
4.9 g. or more of other unsap components before saponification (by calculation)
1.66 g. or less glycerol (by calculation)
Line 5
Total 83.4 g. or more unsap + unsap esters before saponification (by calculation)
and therefore
16.6 g. or less glycerides before saponification (by difference from value opposite)

FIGURE 1. The composition of dogfish liver oil PR3.

esterified "unsap" the vitamin A and cholesterol, calculated as oleates, correspond to 1.26 and 2.14 g. respectively. This remaining portion of the "unsap" therefore becomes on esterification at least 1.5 + 1.26 + 2.14 = 4.9 g. and might be more, depending upon the identity of the 1.5 g. of undetermined material. Line 5 shows the steps which lead to the conclusion that the maximum amount of glycerol combined in 100 g. of oil PR3 can not exceed 1.7 g. This corresponds

with the value 0.96 g. actually found, when the assumptions made are taken into consideration.

It would seem that in dogfish liver oil glyceryl ethers displace glycerol, and therefore that, where these ethers make up a considerable portion of the "unsap", an increasing "unsap" content should result in a decreasing glycerol content. More accurately, an increasing glyceryl ether content should result in a decreasing glycerol content. To check the validity of this hypothesis, the "unsap", glyceryl ether, and glycerol yields from a series of oils from individual dogfish livers were determined. Unfortunately, insufficient of the oil yielding 6.73 per

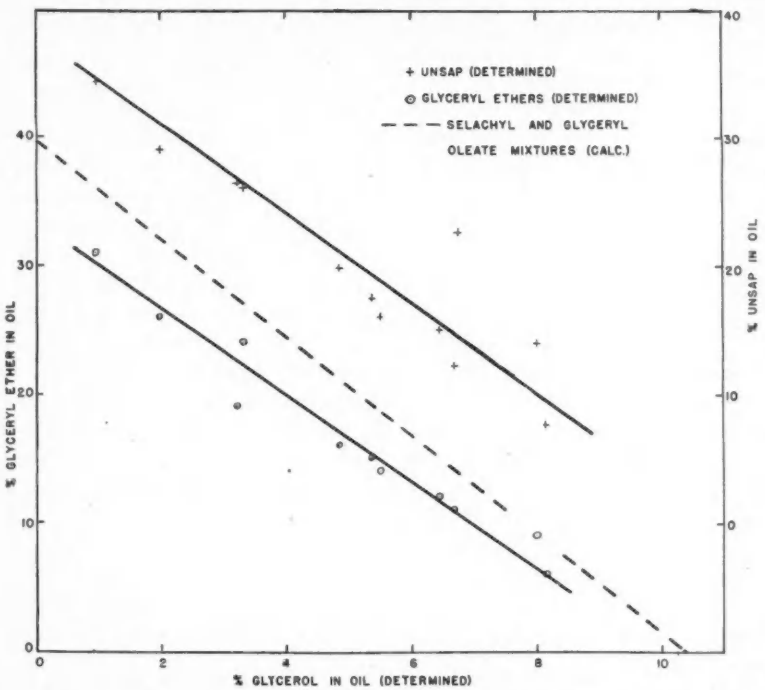


FIGURE 2. Relationship of glycerol yield to "unsap" and to glyceryl ether yields from dogfish liver oils (determined), and to selachyl alcohol yield from selachyl and glyceryl oleate mixtures (calculated).

cent glycerol was available for the glyceryl ether determination. The data are presented in figure 2 and substantiate this hypothesis.

The positions of the curves were determined from a mathematical treatment of the data. For comparison, a curve is shown for similar data calculated for mixtures of glyceryl and selachyl oleates. Its direction is slightly different from those of the other two, which are almost parallel. The different direction is partly due to the fact that not all the ethers in the "unsap" of dogfish liver oil are selachyl alcohol. Decreasing average molecular weight of the ethers causes the direction of this curve to rotate counterclockwise. Its distance from the origin is very similar to that of the "unsap"-glycerol curve. This distance

increases with decreasing average molecular weight of the fatty acids and the direction of the curve moves counterclockwise. The distance also increases with increasing quantities of material other than glyceryl ethers in the "unsap". This fact accounts for the difference in the distances from the origin of the two experimental curves.

Confirmation of the glycerol analyses was obtained by an indirect method, based on the fact that a fat consists of esters, with the possible presence of hydrocarbons, free fatty acids, and substances with uncombined hydroxyl groups. Mono- and di-glycerides, for example, would fall in the latter classification. In the absence of these "impurities", the products of complete hydrolysis of the fat must result in a mixture of substances in which the total hydroxyl content is equivalent to the total replaceable or "acid" hydrogen content. In other words, the ratio OH/H is 17/1. These various products can be separated by saponification followed by ether extraction of the "unsap". The aqueous soap solution then contains glycerol and the soaps of the fatty acids. The "unsap" contains any substances of an alcoholic nature other than glycerol. These would include cholesterol, fatty alcohols, glyceryl ethers, vitamins A and D.

Determinations leading to calculation of hydroxyl and replaceable hydrogen contents were made on those oils recorded in figure 2 which were still available. The hydroxyl content of the "unsap" was determined by the same method. The hydroxyl content of the glycerol was calculated from the per cent of glycerol given in figure 2. The replaceable hydrogen of the fatty acids was calculated from the saponification value of the oil. The results are given in table I. They show fair agreement with theory, indicating that the glycerol determinations are correct. Correspondingly, they offer further substantiation of the hypothesis that glyceryl ethers displace glycerol in dogfish liver oil, for the hydroxyl content due to the one increases as that due to the other decreases.

TABLE I. Hydroxyl and replaceable hydrogen in hydrolyzed dogfish liver oils (mg. per g. oil).

Oil	% "Unsap"	Hydroxyl (OH)			Replaceable hydrogen (H)	OH/H
		Glycerol	"Unsap"	Total		
A16	14.0	44.3	13.8	58.1	3.15	18.4
PR11	15.0	35.8	15.1	50.9	3.04	16.7
PR17	17.5	29.7	18.3	48.0	2.89	16.6
PR10	26.0	18.3	31.7	50.0	2.61	19.2
N6	26.4	17.8	23.9	41.7	2.63	15.9
PR3	34.3	5.3	38.7	44.0	2.36	18.6
					Average	17.6

Free hydroxyl values and free fatty acid values were determined on one of these oils and on other oils of similar history of preparation and storage. The results are shown in table II. Corresponding values should appear respectively as corrections by subtraction in the numerator and denominator of the ratio OH/H for the oils in table I. But as these corrections are in general small in comparison with the values for the saponified oils, they are ignored in this

presentation. It is of interest to note, however, that the highest free hydroxyl value found for an oil was in PR3, the oil highest in "unsap". The corrected OH/H ratio for this oil is $\frac{44.0 - 4.7}{2.36 - 0.006} = 16.7$, a value closer to theory than that given in table I.

TABLE II. Free hydroxyl and replaceable hydrogen in dogfish liver oils (mg. per g. oil).

Oil	Hydroxyl (OH)	Replaceable hydrogen (H)
N1	1.4	0.012
N2	0.9	0.004
N3	1.4	0.004
N4	0.2	0.004
N5	4.2	0.003
PR1	0	0.006
PR2	2.4	0.007
PR3	4.7	0.006
PR4	1.7	0.008

DISCUSSION

The significance of the yield and composition of the "unsap" from British Columbia dogfish liver oils does not appear to have been emphasized previously. Although the maximum yield of "unsap" from the oils examined was only 34.3 per cent as compared with the much higher values reported for liver oils of some other sharks, these dogfish liver oil "unsaps" contained a large proportion of glyceryl mono-ethers. The presence of such ethers, in contrast to the non-esterifiable hydrocarbons reported as making up a large proportion of the "unsap" of some other shark liver oils, leads to interesting conclusions concerning the nature of the original dogfish liver oil.

The demonstrated absence of any significant amount of free hydroxyl groups in the original oils indicates that the glyceryl ethers are doubly esterified with fatty acids. Since the esterified ether has a molecular weight approximately 2.5 times that of the ether, the amounts of up to 31 per cent of ethers isolated from the oils indicate that up to about 80 per cent of the original liver oil consists of these glyceryl ether di-esters. Other "unsap" constituents, some of which are also presumably esterified in the oil, further increase the percentage of "unsap" derivatives in the original oil and correspondingly further decrease the percentage of true fat (triglycerides) that could be present. This conclusion is confirmed by the glycerol determinations after hydrolysis of some of the oil samples; the percentages of glycerol are low in comparison with the yield of some 10 per cent of glycerol on hydrolysis of a typical fish liver oil triglyceride.

The physiological significance of the occurrence of these glyceryl ether esters in dogfish liver oil and of the great variation in the amount present in the liver oil from different fish is of interest but does not come into this investigation.

CONCLUSION

Glyceryl ether esters, known to contribute largely to the "unsap" of dogfish liver oil, replace the glycerides present in a true fat, and decrease markedly the glycerol yield from this liver oil.

Determination of the ratio of the total hydroxyl to the replaceable hydrogen in a hydrolyzed oil offers an indirect check on the accuracy of the determination of glycerol obtainable from the oil.

METHODS

Glycerol Determination

After trial of several methods in the literature, that described by Smith and Matthews (1940) was selected as the most feasible. In this method the oil is saponified with alcoholic potassium hydroxide and the ethanol removed by evaporation, followed by two evaporations of the soap solution with added portions of water. The soaps are then decomposed with sulphuric acid and the resulting fatty acids hardened in the refrigerator and removed. The aqueous glycerol is filtered, made up to definite volume, and cleared with alkaline copper sulphate. An aliquot is oxidized with potassium dichromate under carefully controlled conditions and the excess dichromate is determined.

Evaporation of the ethanol and water proceeded readily by the use of a temperature-controlled hot plate and a motor-driven L-shaped stirring rod. The oxidized solutions resulting from treatment of 8- to 10-g. samples of oil were diluted to 500 ml. Fifty-ml. aliquots were titrated with N/10 sodium thiosulphate after the addition of excess potassium iodide. A sample of herring oil containing 10.40 per cent glycerol according to the formula

$$\% \text{ glycerol} = 0.0547 \times \text{ester value (Dean 1938, p. 57)}$$

analyzed 10.30 per cent glycerol by this method.

Hydroxyl content of glycerol (mg. per g. oil)

$$= \frac{3(OH)}{C_3H_8O_3} \times \frac{\% \text{ glycerol}}{100} \times \frac{1000}{1} = 5.54 \times \% \text{ glycerol.}$$

Preparation of "Unsap"

The "unsap" was prepared by the S.P.A. method (Cocks 1933) modified by using four extractions instead of the prescribed three.

Hydroxyl Value of the "Unsap"

The method of Malm *et al.* (1942) was employed, with the modification that the reaction mixture was heated in a glass-stoppered flask. Phenolphthalein was used as indicator in titrations. One half g. "unsap" was used for each determination.

Hydroxyl content of "unsap" (mg. per "unsap" from 1 g. oil)

$$= \frac{\% \text{ hydroxyl in "unsap"}}{100} \times \frac{\% \text{ "unsap" in oil}}{100} \times \frac{1000}{1}$$

$$= \frac{\% \text{ hydroxyl} \times \% \text{ "unsap"}}{10}.$$

Free Hydroxyl Value of the Oil

The same method was used as for the "unsap", with 1.0- to 1.5-g. samples of oil.

$$\text{Hydroxyl content of oil (mg. per g. oil)} = \frac{\%\ \text{hydroxyl}}{100} \times \frac{1000}{1}$$
$$= 10 \times \%\ \text{hydroxyl.}$$

Replaceable Hydrogen of Fatty Acids

The saponification value of the oil was determined according to Dean (1938, p. 56).

Replaceable hydrogen of fatty acids (mg. per g. oil)

$$= \frac{H}{KOH} \times \text{saponification value} = 0.0178 \times \text{saponification value.}$$

Acid Value

Free fatty acid in the oil was determined according to Dean (1938, p. 55).

Replaceable hydrogen of free fatty acid (mg. per g. oil)

$$= \frac{H}{KOH} \times \text{acid value} = 0.0178 \times \text{acid value.}$$

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The assistance of Miss J. Vernon, Technician, who prepared all the "unsaps" and determined the acid values of the oils, is appreciated.

REFERENCES

André, E., and A. Bloch. *Compt. rend.*, **195**, 627-629, 1932.

Brocklesby, H. N. *et al.* *Bull. Fish. Res. Bd. Can.*, **59**, 1-442, 1941.

Cocks, L. V. *Analyst*, **58**, 203-211, 1933.

Dean, H. K. Utilization of fats. 1-292, Westman Publications, Toronto, 1938.

Hickman, K. *Ann. Rev. Biochem.*, **12**, 353-396, 1943.

Malm, C. J., L. B. Genung and R. F. Williams. *Ind. Eng. Chem., Anal. Ed.*, **14**, 935-940, 1942.

Smith, L. B., and H. Matthews. *Oil and Soap*, **17**, 58-61, 1940.

Toyama, Y., and M. Takahasi. *J. Chem. Soc. Japan*, **60**, 1177-1180, 1939. (*Chem. Abst.*, **36**, 6825, 1942.)

Toyama, Y., and T. Tsuchiya. *J. Soc. Chem. Ind. Japan*, **30**, 207-215, 1927. (*Chem. Abst.*, **21**, 1719, 1927.)

Tsujimoto, M. *J. Ind. Eng. Chem.*, **8**, 889-896, 1916.
J. Ind. Eng. Chem., **12**, 63-72, 1920.

Effect of *Triaenophorus* on Growth of two Fishes

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ABSTRACT

Whitefish parasitized with plerocercoids of *Triaenophorus crassus* are slightly shorter and considerably lighter than non-parasitized fish of the same age. Heavily parasitized tullibee (*Leucichthys* sps.) grow slightly more slowly than lightly parasitized fish.

Whitefish are more affected by the parasites than tullibee, a fact which favours the argument that *Leucichthys* sps. are the more natural hosts for *T. crassus*.

The cestode genus *Triaenophorus* was first reported in Canada by Cooper (1918). It was studied later in Manitoba by Nicholson (1932), Newton (1932) and Wardle (1932), and, in eastern Canada, by Ekbaum (1935, 1937). Recently the life histories of tapeworms of this genus have been investigated in Alberta by Miller (1943a, 1943b and 1945).

Triaenophorus crassus Forel, the most common species, is a tapeworm of considerable economic importance in Canada. The plerocercoids of this parasite encyst in the muscles of the coregonine fishes of many of our best commercial lakes. In this paper the effect of the encysted plerocercoids on the growth rate of the two common hosts in Lesser Slave lake is considered. The fish commonly known as tullibee in this lake (*Leucichthys tullibee* (R)?) are over ninety per cent infested with an average of nine cysts per fish; the whitefish (*Coregonus clupeaformis* Mitchill) are 30 per cent infested with an average of three cysts per fish.

Table I. The average standard lengths of certain age groups of tullibee lightly, intermediately and heavily infested with plerocercoids of *T. crassus*.

Age group (years)	Fish examined (no.)	Average standard length (mm.)			Percent by which heavily infested fish differ from lightly infested
		light (0-5 cysts)	medium (6-15 cysts)	heavy (over 15 cysts)	
3	22	268.0	262.0	—	—
4	37	277.6	272.1	269.0	− 3.10
5	71	285.4	280.6	283.1	− 0.81
6	88	292.8	292.6	290.5	− 0.79
7	74	303.7	301.6	297.9	− 1.93

In table I data are presented for 292 tullibee collected at Lesser Slave lake during December, January and February of 1943-44. The number of cysts of *T. crassus*, the age and the standard length were determined for each fish. In

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the table these data are summarized to show the average standard length of each age for lightly, intermediately and heavily infested fish. The classification light, medium and heavy is, of course, an arbitrary one; it serves, however, to illustrate that the growth rate decreases with an increased burden of parasites. Thus in all the tullibee, with the exception of the five-year-olds, the average length is least in the heavily infected group and greatest in the lightly infected group.

The last column in table I shows the per cent differences in standard length between the lightly and heavily parasitized groups. The most striking fact brought out by these figures is the extreme smallness of the effect of the parasites

TABLE II. The average total lengths and the average weights of certain age groups of whitefish parasitized and not parasitized with plerocercoids of *T. crassus*.

Age group (years)	Fish examined (no.)	Average total length (mm.)			Average weight (gm.)			Percent by which parasitized fish vary from non-parasitized	
		Clean	Parasitized	8 or more cysts	Clean	Parasitized	8 or more cysts	Length	Weight
2	1	350	—	—	624	—		—	—
3	52	394	392	386	805	850	840	− 0.51	+ 5.6
4	203	427	434	429	1320	1130	1050	+ 1.64	− 14.4
5	106	457	450	455	1400	1350	1245	− 1.53	− 3.6
6	40	473	465	465	1540	1495	1540	− 1.69	− 2.9
7	34	480	475	445	1620	1605	1390	− 1.04	− 0.9
8	39	488	485	477	1760	1740	1640	− 0.61	− 1.1
9-11	15	511	503	498	2070	1955	1810	− 1.56	− 5.5

on the growth; for all ages of fish with 16 or more cysts the growth rate is diminished by an average of only slightly over one per cent.

In table II data are presented for 490 whitefish taken from Lesser Slave lake in September 1944. The weight, the total length and the number of cysts per fish were recorded by provincial officials under direction of the Fisheries Research Board of Canada. The author determined the ages of these fish from scale samples collected at the same time. These data are arranged in the same fashion as those for the tullibee in table I with the exception that here it was possible to compare the growth of non-parasitized fish to that of parasitized fish. This was not possible with the tullibee as too few were free of parasites to give reliable figures for growth of non-parasitized fish. To show the effect of heavier infestation the average lengths and weights of the whitefish having eight or more cysts each are shown in the table.

The whitefish are affected by *T. crassus* cysts very much like the tullibee; with the exception of the four-year-olds in length and the three-year-olds in weight the parasitized fish grew less rapidly in length and weight than the clean fish. In general, fish with eight or more cysts grew more slowly than the average for all parasitized fish; these latter figures are not as trustworthy as the others as only 28 fish out of the 490 had eight or more cysts each.

The last two columns of table II show the per cent difference in total length and weight between the parasitized and the non-parasitized fish. The differences in length are small, much the same as in the tullibee; the differences in weight are greater, the parasitized fish weighing from 0.9 to 14.4 per cent less than the non-parasitized.

Cross (1935) studied the effect of parasitism on the growth of perch from three lakes in the Trout lake region of Wisconsin. He classified the perch into three groups, light, medium and heavy infestations, on the basis of their total parasite burden (parasites of several species). He found the heavily parasitized group were as much as 27 per cent shorter and 120 per cent lighter than the lightly infested group. He also demonstrated a greater effect in the older fish than in the younger. The present study agrees with these findings in that a definite diminished growth with increased parasites has been found; the extent of the effect is much less than in Cross's perch. It is possible that the degree of infestation is less in the whitefish and tullibee; a few whitefish which were very heavily parasitized showed effects greater than the average, e.g. a seven-year-old fish with 152 cysts was 16.8 inches long (42.7 cm.) and weighed 38 ounces (1.08 kg.); this fish was 11.1 per cent shorter and 33.4 per cent lighter than the average for non-parasitized fish—figures which approach those reported by Cross.

In Cross's paper no perch older than four-year-olds are included in his tables; he showed that parasitized four-year-old fish were slowed in growth to a greater degree than parasitized three-year-olds. The same can be demonstrated for four-year-old whitefish and tullibee in tables I and II. At this age the maximum retarding effect takes place. With older fish, the degree of the effect does not increase further but diminishes and becomes nearly constant; it is possible that a resistance to the effects of parasitism is acquired as the fish get older.

It is a biological axiom that the perfect parasite is one which does not disturb its host. If this is true it follows that where a parasite has several alternative hosts the one which it least affects is the original host. The author has shown from other considerations (Miller 1944) that *Leucichthys* sps. are the natural hosts for *Triaenophorus crassus* and the whitefish is only an incidental host. Examining the data in tables I and II it can be seen that the tullibee are less affected by *T. crassus* than are the whitefish, since whitefish with only eight or more cysts per fish show as much as five per cent decrease in length while tullibee with 16 or more cysts per fish (twice as many) are not affected as much as five per cent. These facts lend support to the belief that *Leucichthys* sps. are the original hosts for T. *crassus*.

CONCLUSIONS

In conclusion it can be said that the presence of cysts of *Triaenophorus crassus* in the flesh of whitefish and tullibees causes a slight decrease in their

growth rate; the decrease in weight is more pronounced than the decrease in length. The effect is greater the greater the parasite burden. The effect is greatest in fish four years old; in older fish it is less and becomes approximately steady as if the fish acquired a resistance to the parasites. Whitefish, being 'incidental' hosts are more affected by the cysts than the tullibee which are the natural hosts.

REFERENCES

COOPER, A. R. North American pseudophyllidean cestodes from fishes. *Illinois Biol. Monog.*, **4**, 289-541, 1918.

CROSS, S. X. The effect of parasitism on growth of perch in the Trout lake region. *J. Parasit.*, **21**, 267-273, 1935.

EKBAUM, E. Notes on the species of *Triaenophorus* in Canada. *J. Parasit.*, **21**, 260-263, 1935.

On the maturation and hatching of the eggs of the cestode *Triaenophorus crassus* Forel from Canadian fish. *J. Parasit.*, **23**, 293-295, 1937.

MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave lake, Alberta. I. Introduction and the life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the definitive host, *Esox lucius*. *Can. J. Res.*, **21**, 160-170. 1943a.

Ibid. II. The eggs, coracidia and life in the first intermediate host of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas). *Can. J. Res.*, **21**, 284-291, 1943b.

Suggestions for experiments in the control of the pike-whitefish tapeworm, *Triaenophorus crassus*. *Fisheries Serv., Dep. Lands Mines, Prov. Alberta*, 1944.

Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave lake, Alberta. III. Notes on *Triaenophorus nodulosus* (Pallas) in the second intermediate host. *Can. J. Res.*, **23D.**, 1-5, 1945.

NEWTON, M. V. B. The biology of *Triaenophorus tricuspidatus*, (Bloch 1779), in western Canada. *Contr. Canad. Biol. Fish.*, (N.S.) **7** (28), 341-360, 1932.

NICHOLSON, D. The *Triaenophorus* in the flesh of the tullibee (*Leucichthys*). *Can. J. Res.*, **6**, 162-165, 1932.

WARDLE, R. A. The Cestoda of Canadian fishes. II. The Hudson bay drainage system. *Contr. Canad. Biol. Fish.*, (N.S.) **7** (30), 377-403, 1932.

Factors Affecting Deposition of Smoke Constituents on Fish

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ABSTRACT

Methods are given for the estimation of acids, phenols, acetone and colour in smoked fish products. The concentration of phenols in commercial smoked fish ranges from 1.0 mg. per 100 g. of fish in smoked sardines to 35 mg. in hard-cured herring.

Predrying and low smoke velocities decrease the rate of deposition of smoke constituents on the fish. Although volatile phenols are not directly associated with colour development in smoked fish, the concentration of phenols and depth of colour parallel one another. The smoke constituents are shown to be concentrated in the surface layers of the smoked fish.

A satisfactory method for estimating the amount of smoke deposited upon the fish during the smoking process should be of great assistance in the production of smoked fish of uniform quality, and in evaluating projected improvements in present commercial smoking methods. Under present commercial conditions, the depth of colour of the smoked fish is the usual criterion of the amount of smoke present, but, owing to the extensive use of fish dyes, the colour of the fish may bear little relationship to the concentration of constituents of the smoke. This work is an attempt to correlate the amounts of the simpler of these wood-smoke constituents found in smoked fish with the colour of the fish and with the physical conditions under which they are smoked.

Although the chemical composition of wood smoke has been studied by Pettet and Lane (1940), very little quantitative work has been done on the identification or measurement of smoke constituents in smoked fish. Griffiths and Lemon (1934) and Lunde and Mathieson (1934) have shown that formaldehyde is present in smoked fish products. The amounts found were small and were not correlated with the smoking conditions.

ANALYTICAL METHODS

The tunnel smokehouse (Linton and Wood unpub.) was used for most of the experimental smoking work. The commercially smoked fish analysed were bought at local stores.

The substance chosen as a measure of the smoke content must be deposited in appreciable amounts during the smoking process, the concentration found in unsmoked fish should be low and constant, and the compounds should be stable under normal conditions of storage up to the time the fish are eaten.

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Formaldehyde

Eegriwe's (1937) test for formaldehyde was made on the steam distillate from a sample of lightly smoked cod fillet, and the result confirms the findings of Griffiths and Lemon that the amount of formaldehyde in smoked fish is small, in the case of cod fillets less than 1 mg. of formaldehyde per 100 g. of fish tissue. Hoffpauir, Buckaloo and Guthrie's (1943) procedure for the estimation of combined formaldehyde was tried on a sample of smoked fillet but the test was negative. The low concentration of formaldehyde clearly made this compound of little use in estimating the amount of smoke deposited upon the fish, and no effort was made to correlate the amount of formaldehyde found in smoked fish with the various smoking procedures. The small amounts of formaldehyde that can be liberated from smoked fish appear to confirm the assumption of Pettet and Lane that the phenols combine with the formaldehyde chemically at the surface of the fish. An equivalent amount of phenol will also be combined but owing to the relatively high phenol content of smoked fish, the difference between the total phenols and the value obtained by analysis should not be significant.

Volatile Organic Acids

As the analysis of wood smoke by Pettet and Lane showed the presence of relatively high proportions of formic, acetic and higher organic acids, smoked fish should contain a measurable amount of these substances. The methods for the determination of volatile organic acids are well established (Assoc. Off. Agric. Chem., p. 167, 1940).

In the procedure used the fish was minced thoroughly, 20 grams placed in a litre flask and 100 ml. of distilled water added. The mixture was heated to boiling to drive off carbon dioxide and after the addition of one to two drops of mineral oil and two ml. of 10 N sulphuric acid, 500 ml. of steam distillate were collected in about 30 minutes and titrated with 0.01 N sodium hydroxide.

Fish muscle in a good state of preservation contains about 20 mg. of volatile acids, calculated as acetic acid, per 100 g., but bacterial action was found to change this amount materially. Hence a reproducible blank acid value for fish was not obtained. Although smoking increased the volatile acids in fish to 80 or 100 mg. of acid per 100 g. of tissue, this criterion of smoke concentration was considered too unreliable and was not used.

Hydrogen-ion concentration of the fish surface increases in the normal two-hour smoking period in the tunnel smokehouse, from about pH 7.0 to about pH 5.0. On storage, the pH of the surface gradually rises to about 6.0 at the end of 24 hours, and to 6.1-6.2 at the end of 48 hours. During this time, the pH of the interior of the smoked fish falls to about 6.3. These values are influenced to some extent by the state of freshness of the fish and the time of smoking, but it is evident that the pH of the surface does not depend solely on the amount of smoke deposited upon the fish.

Volatile Phenols

Although phenols are found in many biological materials, the only investigation of their presence in smoked food products is that of Tucker (1942) on

smoked meat using Gibbs' indophenol test (1927). He found that, dependent on the distance from the surface, smoked meat contains from less than 0.03 to about 1.9 mg. of volatile phenols per 100 g. of meat. In our work, the measurement of phenols has provided a satisfactory measure of smoke content, being found in amounts as high as 35 mg. per 100 g. of fish.

Two reagents were used to measure the phenols in smoked fish, that of Folin and Ciocalteu (1927) and Gibbs (1927) 2-6 dichloroquinonechloroimide. The former was used on the steam distillate prepared as described under the measurement of volatile acidity, and the procedure was that of Wood, Sigurdsson and Dyer (1942), The reagent was standardized with phenol and was checked against standard tyrosin solutions. In some cases, when foaming was excessive, phosphotungstic acid was added to the distillation mixture. Duplicate steam distillations with and without the use of phosphotungstic acid showed that it had no effect on the amount of phenols distilled over.

Gibbs' reagent was used both on the steam distillate and on a water extract of smoked fish. The reagent was standardized with pure phenol in aqueous solution, and the semi-logarithmic standardization curve proved to be a straight line.

Folin's reagent gives a higher phenol content in the samples of smoked fish than does Gibbs' reagent, the ratio being about three to one. Gibbs' reagent reacts with only those phenols in which the position para to the hydroxyl group is unsubstituted, whereas Folin's reagent reacts with all phenolic and many other reducing substances.

The direct extraction of phenols from smoked fish following Tucker's (1942) procedure is more difficult than measurements on the steam distillate. The use of ethyl alcohol in the extraction mixture was omitted, and 20 g. of smoked fish were comminuted in about 480 ml. of water. The extract was filtered and tested following Tucker's method. A standardization curve was prepared by adding known amounts of phenol to fresh fish and extracting the mixture in the Waring blendor. Difficulty was encountered in obtaining the separation of the normal butyl alcohol and aqueous extract. The mixture was usually centrifuged, and the maximum amount of fish that could be extracted with 480 ml. of water was about 20 g.

The phenols in smoked fillets may be estimated by steam distillation to about 0.1 mg. per 100 g. of fish and by direct extraction to about 0.2 mg. per 100 g.

One steam-distillation experiment was carried out at reduced pressure to ascertain if complex phenolic substances in smoked fish are broken down at the temperature of the steam in the usual steam-distillation process. Phenols, 19.2 mg. per 100 g. were recovered when steam distilled at 5 to 10 cm. of mercury, as compared with 20.0 mg. of phenols per 100 g. recovered in the usual procedure at atmospheric pressure. This indicates that phenols are not influenced by the temperature of the steam-distillation process. The most convenient method for measuring phenols is with Folin's reagent. Tucker's method is longer, but nevertheless, gives reproducible results.

Samples of smoked fish were steam distilled with different amounts of added sulphuric acid. It was established that the pH of the distillation mixture should be 3 or less in order to have a constant phenol value in the steam distillate.

When the pH was adjusted to 3, the state of decomposition of the smoked fish had practically no influence on the phenol value. As table I shows, the amounts of volatile phenolic substances present in fresh fish are relatively small and can be neglected in this work. With Folin's test the values for unsmoked fish of good quality average about 0.35 mg. of phenol per 100 g. of fish, while with unsmoked fish of very poor quality they rise to 0.8 mg. per 100 g. Following the modified Tucker procedure, fresh cod muscle gave a blank of about 0.1 mg. phenol per 100 g. Thus the concentration of phenols in either the steam distillate or in the aqueous extract of unsmoked fish is relatively small.

TABLE I. Analyses of steam distillate from fresh and smoked fish

No.	Product	Condition	Smoking process	Total acidity mg. per 100 g. as acetic acid	Phenols mg. per 100 g. as phenol		Acetone mg. per 100 g.
					Folin's reagent	Gibbs' reagent	
1	Cod fillet	Good	None	18.0	0.32	0.08	0.2
2	" "	Spoiled	"	115	0.8	—	0.35
3	" "	Good	Tunnel	98	—	—	—
3	" "	Spoiled	"	30	—	—	—
4	" "	Good	"	—	24.0	—	1.5
4	" "	Spoiled	"	—	20.2	—	1.7
5	Herring	Good	None	—	0.3	—	—
6	Mackerel	"	"	—	1.0	—	—
7	Cod fillet	"	Commercial	—	10.1	—	—
7	" "	Spoiled	"	—	9.8	—	—
7	" " frozen 6 weeks	Good	"	—	10.0	—	—
8	Cod fillet	"	"	—	6.7	—	—
9	" "	"	"	—	6.5	—	—
10	" "	"	"	—	4.4	—	0.67
11	" "	"	"	—	12.2	—	1.45
12	Finnan haddie		"	—	6.0	1.92	0.80
13	Kippered herring		"	—	23.6	—	2.0
14	" "		"	—	12.7	—	—
15	Hard cured herring		"	225	34.0	—	2.0
16	Smoked boneless herring		"	276	14.8	—	1.2
17	Smoked mackerel		"	230	34.0	—	2.0
18	Canned finnan haddie		"	—	3.3	—	—
19	Smoked sardines		"	—	1.0	—	—
20	Old Salt sardines		"	—	2.4	—	0.4
21	Kipper snacks		"	62	11.0	4.5	0.8
22	" "		"	73	3.3	0.77	0.3

In an experiment to determine the recovery of phenol in the steam distillate from a mixture of unsmoked fish, 1.9 mg. of phenol were recovered in 400 ml. of steam distillate from a total added quantity of 2.0 mg., and 2.0 mg. of cresol were recovered from an added 2.0 mg. Hence, the greater part of the phenols is collected in the first 400 ml. of steam distillate.

Phenols, like all other smoke constituents measured, were found only on or

near the surface of the fish. Hence if very appreciable errors in sampling are to be avoided, the changing ratio between surface and mass with variations in the size of the fish or fillets must be taken into consideration. In this work fillets of very nearly equal size were used, and for analysis each whole fillet was macerated and thoroughly mixed before a sample was withdrawn.

Acetone

The method used for the estimation of acetone is a modification of the procedure of Ravin (1936). Acetone tests were carried out on the steam distillate from the acid mixture of the fish prepared as described under the test for volatile acidity. Five ml. of the unknown were mixed with 4 ml. of 30 per cent sodium hydroxide and with one ml. of 20 per cent salicylaldehyde in 95 per cent ethyl alcohol. (Salicylaldehyde from bisulphite compound manufactured by Eastman Kodak Company is satisfactory). The tube was heated in warm water for 20 minutes at 45 to 50° C. and then allowed to cool at room temperature for 30 minutes. Readings were then made with the Evelyn colorimeter using the 490 mμ filter. A standardization curve was made with pure acetone and the readings gave a straight line relationship when plotted on semi-logarithmic paper. The method appears to be sensitive to about 0.2 micrograms of acetone in 5 ml. of the solution. Other wood smoke constituents, cresol, formic acid, acetic acid, formaldehyde, methyl alcohol gave no colour with the reagents. As is shown in the table, the acetone value for fresh fillets varies from 0.2 to 0.35 mg. of acetone per 100 g. of fish. The amount of acetone found in smoked fish, one to two mg. per 100 g., is relatively small compared with the percentage of phenols found. Hence, acetone measurements are not as accurate for estimating the smoke content.

As is evident from table I, the constituents of phenol and acetone in fish are not appreciably affected by the bacterial decomposition which occurs during prolonged storage.

Colour Measurement

Attempts were made to estimate the colour of smoked fish colorimetrically by extraction of the fish with various organic solvents. Samples of cod fillets were treated in the Waring blendor with petroleum ether, carbon tetrachloride, chloroform, ethyl alcohol, acetone, benzene, 1-4 dioxane, and butyl alcohol but the amount of smoke colour extracted in every case was small and bore no relationship to the colour of the sample.

Much more promising results were obtained by digesting a sample of smoked fish with aqueous potassium hydroxide solution, and measuring the colour of the clear solution in the Evelyn colorimeter. The final method developed consisted in the digestion of a ten-gram sample of the smoked fish with 75 ml. of 8 per cent potassium hydroxide on the steam bath for two hours. The mixture was diluted to 200 ml., and portions of the filtrate measured with the Evelyn colorimeter. Samples of smoked fillets processed for ¾, 1¾, and 2¾ hours in the tunnel smokehouse were digested as described above, duplicate samples were steam distilled, and the phenols measured with Folin's and with Gibbs' reagents. Readings were made in the Evelyn colorimeter with the 420 and 490 mμ filters

and were plotted on semilogarithmic paper against the phenol content (fig. 1). In each case the curve appears to be a straight line indicating that the colour parallels the phenol content of the digested samples. The colour fades slowly on standing, and it must be measured within an hour or two after digestion to obtain its full value. This method offers a means for estimating the colour of smoked fish providing that fish dyes are not used. The method is applicable only to light-coloured fish such as cod or haddock. As figure 1 shows, the method is not particularly accurate, but it is about as accurate as visual examination, and what is much more important, it provides a fixed standard by means of which results of experiments conducted at different times can be compared.

EFFECT OF SMOKING VARIABLES ON SMOKE DEPOSITION

Various factors may influence the rate of deposition of smoke on fish. Previous experiments of Cooper and Linton (unpublished) of this laboratory showed a relationship between smoke velocity and the colour of the product. The trade generally hold that fish will not smoke properly unless dried to a weight loss of four to six per cent previous to their contact with smoke. There appeared to be a definite difference in the smokes produced by the two types of smoke generators used (Linton and Wood unpub). The colour development, after

TABLE II. Effect of variation in smoking conditions on phenol content of cod fillets

Fillet	Storage previous to analysis	Brining time (min.)	Drying time (hours)	Smoking time (hours)	Phenol mg. per 100 g. (Folin method)
A	0	1½	0	1	9.3
A	12 hours	1½	0	1	8.9
A	17 days	1½	0	1	9.4
B	0	1½	1½	1	5.1
B	12 hours	1½	1½	1	5.1
B	17 days	1½	1½	1	5.2
C	0	1½	0	2	13.5
C	12 hours	1½	0	2	12.0
C	1½ days	1½	0	2	12.9
D	0	1½	1½	2	11.0
D	12 hours	1½	1½	2	9.3
D	1½ days	1½	1½	2	9.4
E	—	None	0	2	13.1
G	—	1½	½	2	12.3

two hours' smoking in the tunnel, does not reach a maximum until about ten hours at room temperature. Therefore, a number of smoking experiments were made, both with whole cod fillets and with small cut portions, to determine the relationship between the various factors such as predrying, time of smoking, velocity of smoke, and type of smoke on the amounts of phenols deposited on fish.

The results of experiments with smoked fillets are summarized in table II. These fillets were subjected to smoke from the cone smoker, at a dry-bulb temper-

ature of 23° C., a relative humidity of 58 per cent, and a smoke density of 0.30. They show that the phenol content remains constant for at least 17 days at 20° C. During this period the fish assumed its full colour. Hence, the phenols do not enter directly into the final colour reaction. On the other hand, while they are not associated directly with this colour development, the final colour is roughly proportional to the concentration of phenols. A comparison of fillets C and E shows that brining does not affect the deposition of phenols. Predrying for 1½ hours actually lessens the phenol content. This may be due in part to the drier surface in which phenols dissolve less readily, and in part to a warmer surface on which the smoke is not so rapidly deposited. The relationship between phenol deposition and smoking time is well demonstrated. The deposition is not directly proportional to the time but falls off as the smoking proceeds.

TABLE III. Analyses of cut samples smoked under different conditions

Sample number	Smoking time (hours)	Mean smoke density	Storage period	Method of removal of smoke products	Phenol (mg. per sq. cm.)		Acetone (mg. per sq. cm.)	Colour
					Folin's reagent	Gibbs' reagent		
1†	2	0.28		—	0.057	—	0.0075	Medium
2*†	2	0.35		—	0.042	—	0.0052	Light
3***	2	0.26		—	0.053	—	—	Medium
4	1	0.21	0	Extraction	—	0.0040	—	—
4	1	0.21	3 days	"	—	0.0050	—	—
4	1	0.21	0	Distillation	0.036	0.011	0.0025	—
4	1	0.21	3 days	"	0.035	0.0094	0.0035	—
5	2½	0.21	0	Extraction	—	0.0081	—	—
5	2½	0.21	3 days	"	—	0.0083	—	—
5	2½	0.21	0	Distillation	0.039	0.013	0.0023	—
5	2½	0.21	3 days	"	0.041	0.012	0.0035	—
6**	2½	0.21	0	"	0.013	0.0051	—	—

*Smoke velocity 75 cm. per sec., for all others 225 per sec.
†Relative humidity of smoke 70 per cent, for all others 55 per cent.
**Predried for 1.5 hours, all others were not predried.
***Smoked with the Smokemaster, all others with the cone smoker.

Since in the previous experiments whole fillets probably varying considerably in surface were used, a new series of experiments was set up, using small cut pieces of cod tissue of known dimensions. These pieces, cut from cod fillets with a razor, were 10 to 11 cm. long, 3 to 4 cm. wide, and 7 to 8 mm. thick. They were weighed on glass plates and so embedded in paraffin that one surface only was exposed to the smoke. The analyses of these cut samples, which had been smoked under various conditions, are shown in table III. All samples were smoked by the Smokemaster with the exception of no. 3, which was smoked with the cone smoker. The smoke velocity with sample no. 2 was 75 cm. per second, all others about 225 cm. per second. Relative humidities for samples nos. 1 and 2 were 70 per cent, for all others 55 per cent.

The results confirm those of the previous series. Drying previous to smoking reduces the amounts of both phenols and acetone deposited. Both the concentration of phenols and the final colour increase with the time of smoking. Adequate colour appears to correspond with a phenol deposition of 0.06 mg. per sq. cm. as measured by Folin's reagent, or an acetone value of 0.005 mg. per sq. cm. These values are for an average smoke velocity of 200 to 250 cm. per second and an average smoke density of 0.30. The experiments show the effect of increasing the smoke velocity. At 200 to 250 cm. per second the phenol deposition was 0.057 mg. per sq. cm., while at 75 cm. per second it fell to 0.042 mg. per sq. cm. The smoke velocity in commercial smokehouses is about 25 cm. per second. Hence the low rate of smoking.

The high temperature used in the steam distillation procedure for the estimation of the phenols produces full development of colour in the smoked fish sample before the distillation is completed. Hence if the concentration of the phenols changes during colour development, this will not be detected by the analysis of the distillate. The aqueous extraction procedure for phenols is not open to this objection as it is carried out at room temperature. The analyses of samples 4 and 5 show that the same amount of phenols is extracted before and after full colour development. This is good evidence that the phenols do not polymerize or combine chemically with other smoke constituents as the colour deepens. Presumably, the colour in smoked fish comes from resins or tars in the smoke and the colour develops by a process of polymerization or auto-oxidation.

A comparison of cut samples, nos. 1, 2 and 3, shows that the type of smoker has little or no influence on the amounts of phenols (Folin) deposited on the fish. Analysis of smoked fish for acetone and phenols (Gibbs) also shows no difference in the samples smoked by means of the cone smoker and by the Smokemaster.

The results of an experiment, in which samples of smoked cod fillets of different phenol content were digested with potassium hydroxide, and colour measured, are shown in figure 1. It will be noted that the optical density of the solution is proportional to the amount of phenols present. Predried samples, however, have a deeper colour than the undried samples for the same phenol content. This is the first quantitative evidence that the colouring substances in the smoke do not parallel the simpler chemical constituents under all variable physical smoking conditions. It would appear that the dried surface of the predried samples does not take up the volatile chemical constituents as readily as do the undried samples, but that the colouring substances are deposited quite irrespective of the amount of moisture present. The effect is more pronounced during the initial smoking period, and practically disappears after two or three hours' smoking. A sample of smoked fish was digested and portions of the digested mixture were steam distilled periodically over the course of several days to determine if there was any variation in phenol content. The original sample had a phenol content of 21.3 mg. per 100 g. Immediately after digestion this fell to 20.7 mg. and gradually decreased, as fading of the colour progressed, to 17.0 mg. Gibbs' test on the steam distillate gave an essentially constant value of 6.3 mg. of phenols per 100 g. throughout the aging period. It appears that the alkali digestion does not decompose the phenols nor polymerize them during

the fading process. This is further confirmation of the fact that the simpler volatile chemical constituents of smoked fish are not associated directly with the colour.

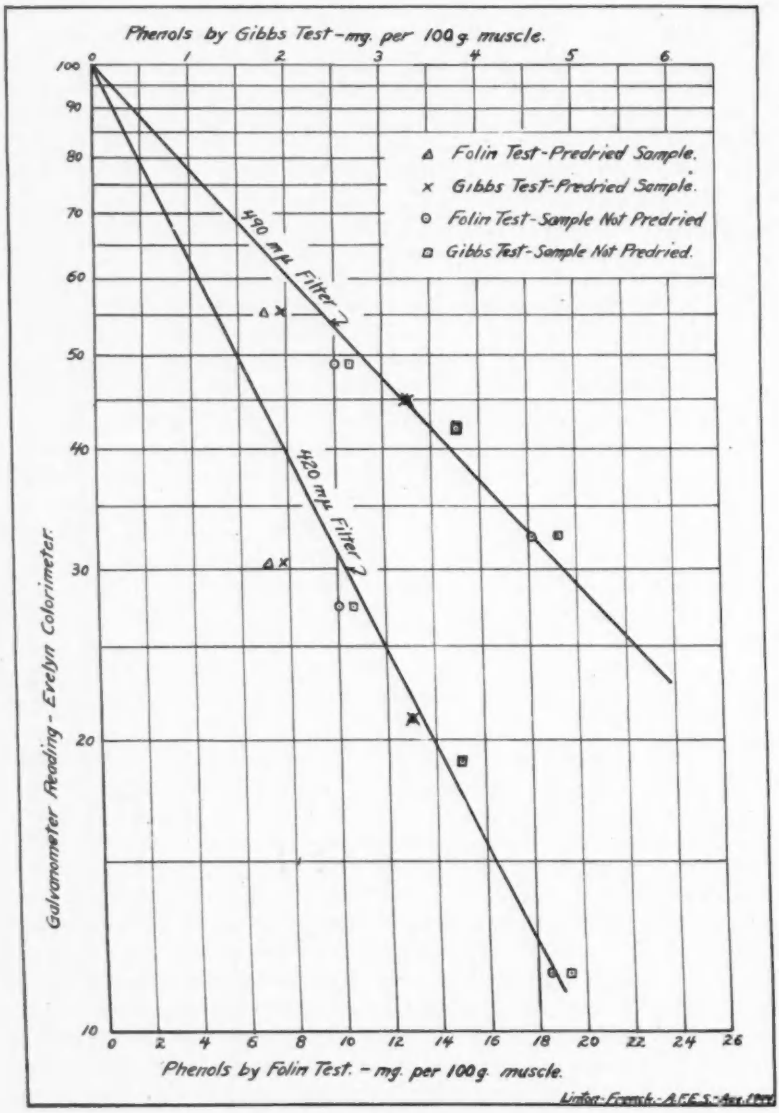

FIGURE 1. Relation between phenol content and colour of digested samples of smoked cod fillets.

In another experiment a sample of smoked fillet was digested and the colour measured. A sample of the digested mixture was then acidified and steam dis-

tilled to remove volatile acids, phenols, and acetone, after which the residue from the steam distillation was made alkaline to give the original concentration of alkali. When the colour of this sample, from which the volatile phenols, acetone and acids had been removed was measured, it gave essentially the same value as the original digestion mixture. This again confirms the fact that the colour is not associated with the known volatile chemical constituents present.

DISTRIBUTION OF PHENOLS IN SMOKED COD FILLETS

There is some difference of opinion on the extent to which smoke penetrates into fish. An examination of commercial fillets indicates that the fish dyes diffuse uniformly to a depth of about one cm. in smoked cod fillets. In the case of the smoke, however, a layer a few mm. thick in the surface appears to contain a greater part of the colour in smoked fish. Tucker has shown that in the smoking of meats the phenols remain concentrated in the surface layer. A fillet smoked for about two hours in the tunnel smokehouse and then stored for about a month at − 16° C. was sliced into layers 2 mm. in thickness, and each layer was analysed for volatile phenols using Folin's reagent.

TABLE IV. Penetration of phenols into smoked cod fillets

Distance measured from surface (mm.)	Mg. phenols per 100 g. fish
0- 2	25
2- 4	14
4- 6	11
6- 8	7.0
8-10	5.0
Centre section	3.1

It is evident from the results (tab. IV) that the greater part of the phenols are concentrated in a 4 mm. layer at the surface of the smoked fish. This fact corroborates a visual examination of smoked fish. The centre sections of smoked fillets have little colour and a low phenol content, the amounts depending upon the extent of the smoking process.

SUMMARY

The constituents of wood smoke most convenient and reliable for measurement of the rate and extent of smoke deposition on fish are the phenols. They increase with the other smoke constituents, and are fairly readily measured. They increase with the yellow smoke colour but they appear not to be associated directly with colour development. The analyses show that smoke deposition is only on the surface of the fish. Predrying is not necessary if the wood smoke is sufficiently dry and moving rapidly over the fish. While the rate of deposition of phenols is not directly proportional to, it increases with the smoke velocity. Cod fillets or haddock can be smoked adequately in less than three hours, being deeper in colour and higher in phenols than the greater part of these produced by the trade.

REFERENCES

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Methods of analysis. Fifth ed., Washington, D.C., 1-757, 1940.

EEGRIWE, E. *Z. Anal. Chem.*, **110**, 22-25, 1937.

FOLIN, O., AND V. CIOCALTEU. *J. Biol. Chem.*, **73**, 627-650, 1927.

GIBBS, H. D. *J. Biol. Chem.*, **72**, 649-664, 1927.

GRIFFITHS, F. P., AND J. M. LEMON. *U.S. Bur. Fish. Invest. Rep.*, **20**, 1-12, 1934.

HOFFPAUIR, C. L., G. W. BUCKALOO, AND J. D. GUTHRIE. *Ind. Eng. Chem., Anal. ed.*, **15**, 605-606, 1943.

LUNDE, G., AND E. MATHIESON. *Ind. Eng. Chem.*, **26**, 974-976, 1934.

PETTET, A. E. J., AND F. G. LANE. *J. Soc. Chem. Ind.*, **59**, 114-119, 1940.

RAVIN, A. J. *J. Biol. Chem.*, **115**, 511-518, 1936.

TUCKER, I. W. *J. Assoc. Offic. Agri. Chem.*, **25**, 779-782, 1942.

WOOD, A. J., G. J. SIGURDSSON, AND W. J. DYER. *J. Fish. Res. Bd. Can.*, **6**, 53-62, 1942.

Bacteriostatic Action of Hydroxylamine

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ABSTRACT

From 0.001 to 0.024% hydroxylamine caused transient or permanent growth inhibition of fish spoilage and certain other bacteria.

Kitasato and Weyl (1890) showed that growth on nutrient agar of certain strict anaerobes of the genus *Clostridium* was entirely prevented by 0.05 per cent of hydroxylamine (NH_2OH). Recently Jensen (1944) has drawn attention to the fact that growth of certain food poisoning *Staphylococci* may be inhibited by 0.005 per cent of NH_2OH hydrochloride. Probably this inhibition is related to the action of NH_2OH on certain bacterial respiratory catalysts, for Sevag and Shelburne (1942) showed that respiration of *Streptococcus pyogenes* is markedly inhibited by NH_2OH, concentrations as low as 0.0033 per cent markedly retarding oxygen uptake of washed suspensions. Sevag, Shelburne and Ibsen (1942) believe that NH_2OH is capable of combining with and inhibiting both heme and non-heme respiratory catalysts. It has long been known that NH_2OH inhibits catalase activity (Jacobson 1892) and the mechanism of this effect has been studied in some detail (Keilin and Hartree 1938).

In view of these facts, and because NH_2OH is possibly formed as an intermediate in the metabolism of nitrate, nitrite and ammonia by bacteria (Kluyver and Donker 1926, Lindsey and Rhines 1932, Woods 1938), the action of NH_2OH on growth of certain heterotrophic bacteria is being studied. It has been found that NH_2OH strongly retards development of fish spoilage bacteria. Thus 0.001 per cent NH_2OH exerted a transient inhibition on growth in nutrient broth at 25° C. of ten different species studied, the inhibition lasting for two weeks in the case of two of the organisms. Growth of eight of the ten cultures was entirely inhibited after two weeks by 0.0025 or 0.005 per cent NH_2OH, while two of them eventually developed in broth containing 0.005 per cent. Development of a strain of food poisoning *Staphylococcus* in nutrient broth at 37° C. was totally inhibited at pH 5.9 after two weeks by 0.0025 per cent NH_2OH but at pH 7.1 and 7.5 the inhibiting effect of 0.005 per cent NH_2OH was purely transient. No growth occurred after two weeks at 37° C. in fish digest broth cultures of type A *Clostridium botulinum* containing 0.005 per cent NH_2OH at pH 6.3 or higher, while at pH 5.9 a 0.0025 per cent concentration prevented multiplication. With certain of the cultures studied, NH_2OH retarded develop-

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ment more strongly in acid than in neutral or alkaline medium but the effect of pH was neither as pronounced nor as universal as in the case of inhibition due to nitrite (Tarr 1941). In minced lingcod muscle stored at 0° C. development of the normal mixed bacterial flora was markedly delayed by 0.0024 to 0.024 per cent NH_2OH. It is intended to publish these results more fully in a subsequent issue of this Journal.

REFERENCES

JACOBSON, J. *Z. Physiol. Chem.*, **16**, 340-369, 1892.
JENSEN, L. B. *Bact. Rev.*, **8**, 161-188, 1944.
KEILIN, D., AND E. F. HARTREE. *Proc. Roy. Soc. (Lond.)*, B**124**, 397-405, 1938.
KITASATO, S., AND T. WEYL. *Zeit. f. Hyg.*, **8**, 41-47, 1890.
KLUYVER, A. J., AND H. J. L. DONKER. *Chem. Zelle Gewebe*, **13**, 134-190, 1926.
LINDSEY, G. A., AND C. M. RHINES. *J. Bact.*, **24**, 489-492, 1932.
SEVAG, M. G., AND M. SHELBURNE. *J. Bact.*, **43**, 421-445, 1942.
SEVAG, M. G., M. SHELBURNE AND M. IBSEN. *J. Biol. Chem.*, **144**, 711-717, 1942.
TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **5**, 265-275, 1941.
WOODS, D. D. *Biochem. J.*, **32**, 2000-2012, 1938.